PRENATAL EXPOSURE TO NITRATES, NITRITES, NITROSATABLE DRUGS, AND SMALL-FOR-GESTATIONAL-AGE BIRTHS

A Dissertation

by

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ABSTRACT

Certain drugs, which contain nitrosatable amines (secondary or tertiary amines) or amides can react with nitrite in the stomach to form N-nitroso compounds. Experimental data from animal studies suggest that exposure to these compounds might reduce fetal birthweight. We examined the relation between prenatal exposure to drugs classified as nitrosatable and dietary intake of nitrates/nitrites and small-for-gestational (SGA) births. Data were analyzed from the National Birth Defects Prevention Study (NBDPS) control participants (mothers of babies without major birth defects), that included 526 mothers who delivered infants with birthweight <10th percentile and 5970 mothers of control infants (birthweight ≥10th percentile for gestational age) during 1997-2005. Information was collected by telephone interview on type and frequency of medication use, diet, supplementation, demographic characteristics, and maternal health. Overall, prenatal use of nitrosatable drugs was not associated with SGA except for a few notable exceptions. Relative to women who reported no nitrosatable drug use anytime during pregnancy, women who took nitrosatable amides during the third trimester of pregnancy were more likely to have SGA births (adjusted odds ratio [OR] 1.4 [95% confidence interval [CI] 1.0, 2.1]). This association was stronger among full term SGA births (OR 1.6 [95% CI 1.1, 2.3]). Dietary nitrites modified the associations between nitrosatable drugs and SGA but lower odds of SGA were observed among women with higher nitrite intake. Higher intake of dietary vitamin C (≥85 mg/day) in combination with

daily vitamin C supplementation reduced the associations between SGA and secondary amine use during the second trimester of pregnancy (aOR 1.0 [95% CI 0.65, 1.6]) compared with <85mg of dietary vitamin C and less than daily use of vitamin C supplement (OR 4.0 [95% CI 1.5, 10.9]). Prenatal exposure to nitrosatable drugs and higher intake of dietary nitrites did not appear to be associated with SGA. Supplemental and dietary vitamin C may modify the risk of SGA birth in relation to nitrosatable drug use during pregnancy.

DEDICATION

To my parents and husband for believing in my dreams and helping me accomplish them.

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NOMENCLATURE

AP	Attributable proportion due to interaction
BMI	Body mass index
CBDRP	Centers for birth defects research and prevention
CI	Confidence interval
CO	Carbon monoxide
EDD	Estimated date of delivery
FFQ	Food frequency questionnaire
GDM	Gestational diabetes mellitus
IUGR	Intrauterine growth restriction
NBDPS	National Birth Defects Prevention Study
NDMA	N-nitrosodimethylamine
NO_2	Nitrogen dioxide
NRT	Nicotine replacement therapy
O_3	Ozone
OR	Odds ratio
PAH	Polyaromatic hydrocarbons
RERI	Relative excess risk due to interaction
SGA	Small for gestational age
SIMEX	Simulation extrapolation
SO_2	Sulphur dioxide
SSRI	Selective serotonin reuptake inhibitors

USDA	United States Department of Agriculture

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1. INTRODUCTION

Birthweight is regarded as the single most important determinant of an infant's survival and subsequent health. In 2009, the infant mortality rates were 24 times higher (53.05 per 1,000) for low birthweight (less than 2500 grams) infants than for infants with birthweight 2500 grams or more.[1] Infants born small for gestational age (SGA) are also at increased risk of mortality during the first year of life. SGA is usually defined as infants whose birthweight or crown-heel length is less than expected for their gestational age and sex.[2] These infants may have a persistent short stature in childhood and adulthood. However, most infants present early postnatal or catch-up growth from birth to two years of age, and rapid weight gain during childhood. SGA infants are also at higher risk of developing poor cognitive and neurologic disorders in childhood and chronic diseases in adulthood such as cardiovascular disease, insulin resistance, diabetes mellitus, dyslipidemia, and renal disease.[3-6]

Size at birth is dependent on the fetus's trajectory of growth and the maternal placental capacity to supply sufficient nutrients to maintain that trajectory. Evidence suggests that reduced substrate delivery to the fetus caused by placental insufficiency or poor maternal nutrition may play a role in the etiology of small-for-gestational-age (SGA).[7] Several aspects of maternal diet have been hypothesized to influence fetal growth including dietary patterns;[8-10] vegetable and fruit consumption;[11-13] and Vitamin C and folic acid

supplementation.[12, 14-16] Kwong et al. demonstrated that low protein diet fed to pregnant rats during the preimplantation period induced structural and functional abnormalities in organ and tissue development, and reduced fetal growth rate and birthweight in offspring.[17] Small variations in maternal dietary patterns during early pregnancy have been associated with a reduced risk of SGA birth.[18] However, limited studies have examined the association between dietary consumption of nitrates and nitrites and SGA.

Exposure to nitrates and nitrites can occur from diet, drinking water, certain medications, and environmental or occupational sources of which dietary consumption accounts for a significant portion of daily nitrite exposure.[19] Nitrates are commonly found in vegetables and root crops; and nitrite is used for curing of meat products. Approximately 5% of the nitrates ingested are converted to nitrite in the saliva and a portion of the nitrite is further reduced to nitric oxide in the stomach.[20, 21] Nitric oxide is also produced endogenously by endothelial cells from L-arginine and it plays an important role in implantation, embryo development, and placental vascular development.[22] On the other hand, higher nitric oxide levels have been shown to arrest embryo development and alterations in nitric oxide production may impact fetal growth.

N-nitroso compounds might also contribute to fetal growth restriction. These compounds are formed when nitrosating agents such as ingested nitrates and nitrites react with nitrosatable amine (secondary or tertiary amine) or amide containing drugs in the acidic environment of the stomach.[23, 24] A variety of

prescription and non-prescription drugs, which contain secondary or tertiary amines or amides, are commonly used during pregnancy. Approximately 24 percent of the control mothers in the National Birth Defects Prevention Study (NBDPS) reported use of one or more drugs classified as nitrosatable during the first trimester of pregnancy.[25] N-nitroso compounds are known to cause adverse pregnancy outcomes such as congenital malformations and reduced fetal weight in animal models. The role of these compounds on fetal growth and risk of SGA birth needs to be further examined.

The long-term goal of the proposed study was to examine whether dietary nitrate or nitrite intake and nitrosatable drug use is associated with SGA births. The objective of this study was to investigate the independent and joint effects of dietary nitrite or total nitrite and nitrosatable drugs on risk of having SGA infants and the role of Vitamin C, a known nitrosation inhibitor, in lowering this risk. Data was used from the NBDPS control mothers who delivered infants without major congenital malformations with estimated dates of delivery from 1997 to 2005.

Specific aims were:

1. To assess whether nitrosatable amine or amide drug use during pregnancy increases the risk of SGA births (Chapter II).
   Drugs were classified into secondary or tertiary amine, and amide depending on nitrosatability, functional group, and indication.

2. To examine the association between maternal dietary consumption of nitrite/total nitrite (dietary nitrite + 5% dietary nitrate) during pregnancy and risk of SGA birth (Chapter III).

   Daily nitrite and total nitrite intake were estimated based on the nitrate and nitrite content in each food item per serving size and frequency of intake.

3. To determine if joint effects of nitrosatable drug and dietary nitrite or total nitrite intake increases the risk of SGA birth (Chapter III).
4. To evaluate the effect of dietary or supplemental Vitamin C on the relation nitrosatable drug use during pregnancy and SGA (Chapter IV).

The causes and mechanisms of SGA are multifactorial. Several maternal risk factors have been identified to be associated with SGA. Kramer reviewed approximately 900 published studies and categorized maternal risk factors identified to be associated with intrauterine growth restriction (IUGR) into following groups: genetic and constitutional factors (maternal height, prepregnancy weight); obstetrical factors (parity, history of low birthweight infant, general morbidity and illness, malaria); nutritional factors (gestational weight gain); and substance abuse factors (cigarette smoking, tobacco chewing, and alcohol consumption).[26] A review of maternal and environmental risk factors associated with SGA is covered below.

Maternal demographic and obstetric factors

Maternal demographic predictors such as height, prepregnancy weight and low gestational weight gain may contribute to risk of SGA birth.[11, 27-29] Lang and colleagues observed that mothers who were less than 5 feet tall had higher risk for SGA (OR 1.9 [95% I 1.5, 2.4]) compared to mothers who were 5'5''-5'7''tall. Those weighing less than 100 pounds prior to pregnancy were twice as likely [95% CI 1.8, 3.2] to have SGA infants compared to those weighing 126-160 pounds.[27] Similar findings were noted by Kramer et al. with regard to maternal risk factors associated with IUGR. Each 10 cm change in maternal height (OR 1.29 [95% CI 1.10, 1.53]), and 10 gram increase in prepregnancy weight (OR 1.65 [95% CI 1.45, 1.87]) was significantly associated with IUGR.[28] In addition, Thompson et al. noted that mothers of SGA infants were significantly shorter (161.5 cm v. 164.8 cm, $p < 0.001$) and lighter (59.8 kg v. 66.5 kg, $p < 0.001$) compared to mothers of appropriate for gestational age infants.[29]

Lower gestational weight gain during pregnancy might also increase the risk of SGA birth.[27, 28, 30] Women who had less than 0.40 pounds of weekly weight gain during pregnancy were three times more likely [95% CI 2.2, 3.6] to have SGA birth compared to those with higher weight gain.[27] Scott et al. noted similar observations among multiparous women; low weight gain during pregnancy elevated the risk of SGA birth (OR 1.78 [95% CI 1.1, 2.8]) and contributed to 10.2% of the total SGA babies in the population.[30] Furthermore,

Kramer et al. found an increase in risk for IUGR (OR 1.32 [95% CI 1.20, 1.44]) with each 5 kg decline in gestational weight gain.[28]

Racial and ethnic disparities have been observed in the prevalence of low birthweight and SGA births. The risk of low birthweight has been consistently higher among Black than White infants, a disparity that has existed for decades.[31-35] Differences in birthweight of offspring have also been reported between US born and African born Black women. Using data from the Illinois vital records from 1980-1995, David & Collins reported that the prevalence of low birthweight was 13.2% among infants born to U.S. born Black women, and 7.1% among African born Black women compared to 4.3% among U.S. born White women (OR 3.1 [95% CI 2.9, 3.2] and OR 1.6 [95% CI 1.4, 1.9], respectively).[32] Kramer et al. noted similar findings in a large cohort of White and Black singleton livebirths in U.S. from 1998-2000.[35] Compared to White women, the risk of SGA births was higher among U.S. born than foreign born Black women (OR 2.05 [95% CI 2.04, 2.06] v. OR 1.49 [95% CI 1.47, 1.51]).

Maternal obstetrical factors that include parity, pregnancy induced hypertension, and history of low birthweight infants have been identified as significant predictors of SGA. Several studies have reported nulliparous women to be at increased risk for SGA birth compared to multiparous women.[11, 27, 29, 36, 37] Previous history of a pregnancy with low birthweight has been associated with a higher risk of having a SGA infant.[30, 36] Pregnancy induced hypertension, especially preeclampsia has known to be associated with IUGR.[28-30, 38] Women

diagnosed with gestational hypertension were 49% more likely to have an infant with IUGR (OR 1.49; 95% CI 1.14, 1.93) compared to those without hypertension.[38] The risk of IUGR increased with severity of preeclampsia, ORs of 1.97 (95% CI 1.43, 2.73) and 1.95 (95% CI 1.14, 3.37), respectively, were noted among women with preeclampsia and severe preeclampsia. Thompson et al. also noted an increased risk for IUGR with increasing severity of pregnancy induced hypertension with ORs ranging from 1.5 to 5.5.[29] Other studies reported similar findings with risk elevated among women who had preeclampsia.[28, 30]

Substance abuse factors

Smoking

Smoking during pregnancy is a known risk factor for IUGR. Several mechanisms have been suggested through which smoking may affect fetal growth.[39] First, carbon monoxide produced from tobacco smoke may cause fetal hypoxia due to increased carboxyhemoglobin levels. Second, nicotine induces an increase in maternal catecholamines and subsequently results in uterine vasoconstriction. Third, cyanide compounds in tobacco smoke may interfere with fetal oxidative metabolism. A positive association between smoking and IUGR has previously been reported.[39-42] Horta et al. examined the association between maternal smoking during pregnancy and risk of IUGR among 5,166 infants born during 1993 in Pelotas, Brazil.[39] Mothers who reported smoking during pregnancy were 2.1 times more likely [95% CI 1.69, 2.53] to deliver an SGA infant. The risk for IUGR doubled with increase in number of cigarettes smoked

(<1 to ≥ 20 cigarettes/day) with ORs ranging from 1.1 to 2.5. In addition, mothers who stopped smoking during the first trimester had slightly lower risk than those who continued smoking until the second or third trimester of pregnancy. Chiolero et al. noted similar findings in a population based cohort of 6,284 singleton births in the Canton of Vaud, Switzerland from 1993-1994.[41] A higher proportion of mothers with SGA infants reported smoking during pregnancy (18.8%) compared to controls (9.9%). The odds of delivering an SGA infant (OR 1.4 [95% CI 1.1, 1.9]) was higher among smokers compared to non-smokers.

Although there is strong evidence that smoking cessation during pregnancy might improve neonatal outcomes such as birthweight but only one in four women may quit smoking during pregnancy.[43] Several studies found that reduction in smoking during pregnancy was associated with an increase in infant birthweight.[44-46] Li et al. reported that mean birthweight of infants born to women who reduced smoking during pregnancy was 92g higher than among women who did no change their smoking behavior during pregnancy.[45]

Furthermore, infants born to women exposed to environmental tobacco smoke are subjected to most of the same toxic compounds as those contained in mainstream smoke, but the pattern and amount of exposure may vary. Studies have shown that prenatal exposure to environmental tobacco smoke elevated the risk of delivering a low birthweight infant.[47-51] However, inconsistent findings have been reported regarding the associations between SGA and maternal exposure to environmental tobacco smoke.

Alcohol intake

The impact of chronic heavy alcohol consumption during pregnancy on fetal growth is well documented. Fetal alcohol syndrome, a characteristic disorder of fetal growth restriction with mental retardation and facial anomalies has been linked with heavy maternal alcohol consumption during pregnancy.[52] However, the effect of light or moderate alcohol intake on fetal growth is unclear. Majority of studies have found no association between prenatal exposure to low levels of alcohol and risk of SGA birth,[53-57] while few studies have reported an increased risk.[42, 58, 59] In a retrospective cohort study, Windham et al. examined the association between moderate maternal alcohol consumption during pregnancy and birthweight.[42] Women who reported an average of three or more drinks per week had increased odds of delivering a SGA infant (OR 2.3 [95% CI 1.2, 4.6]). Using data from the Pregnancy Risk Assessment Monitoring System, Whitehead & Lipscomb noted that heavy drinking (≥ 14 drinks/week) during the last three months of pregnancy was associated with a four-fold risk [95% CI 1.11, 16.11] of SGA birth; however, only 28 women reported heavy drinking during this period.[59] Chiaffarino et al. confirmed findings from the previous studies.[58] Among women who reported three or more drinks/day, the risk of SGA birth was 3.2 [95% CI 1.7, 6.2] from exposure during the first trimester, 2.7 [95% CI 1.4, 5.3] and 2.9 [95% CI 1.5, 5.7], respectively, from exposure during the second trimester and third trimester of pregnancy.

Environmental risk factors

The impact of ambient air pollutants such as sulphur dioxide (SO_2), nitrogen dioxide (NO_2), ozone (O_3), carbon monoxide (CO), polyaromatic hydrocarbons (PAHs), and fine and coarse particulate matter with aerodynamic diameters of ≤ 2.5 μM ($PM_{2.5}$) and ≤ 10 μM (PM_{10}), respectively, and their potential interactive effects on fetal growth has previously been examined; however, inconsistent findings have been reported by different studies.

Sulphur dioxide

While few studies reported positive association between maternal SO_2 exposure and low birthweight,[60-63] other studies found null associations.[64-68] In an ecologic study conducted in Czech Republic, Bobak & Leon observed increased risk for low birth weight with every 50 μg/m^3 increase in exposure to SO_2.[60] Examining exposure to SO_2 by each trimester of pregnancy, Lee et al. noted significant risk for low birthweight among mothers exposed during the second trimester of pregnancy (OR 1.06 [95% CI 1.02, 1.11]).[62] Dugandzic et al. also observed elevated risk for low birthweight with first trimester exposure to SO_2 (OR 1.15 [95% CI 1.00, 1.31]).[61] Further, Liu et al. found that exposure to SO_2 during the first month of pregnancy was significantly associated with low birthweight (OR 1.11 [95% CI 1.01, 1.22] per 5 ppb increase in SO_2 concentration).[63] The risk for IUGR was also higher with SO_2 exposure during the same period (OR 1.07 [95% CI 1.01], 1.33 per 5 ppb increase in SO_2 concentration).

Nitrogen dioxide

The association between NO_2 exposure during pregnancy and SGA has been explored previously. The risk for SGA births was elevated with maternal exposure to NO_2 during the first month of pregnancy (OR 1.05 [95% CI 1.01, 1.10] per 10 ppb increase in NO_2);[63] second trimester (OR 1.37 [95% CI 1.01, 1.85] per 10 $\mu g/m^3$ increase in NO_2 concentrations);[69] and third trimester of pregnancy (OR 1.01 [95% CI 1.00, 1.02]).[70] Conversely, other studies found no increased risk for SGA with NO_2 exposure during pregnancy.[71, 72]

Carbon monoxide and ozone

Most studies have found null findings with exposure to ambient levels of carbon monoxide or ozone during pregnancy.[68, 70, 73] Only two studies noted slightly elevated risk of SGA with CO exposure during the first month (OR 1.06 [95% CI 1.01, 1.10] per 1 ppm increase in CO concentration)[63] and the first trimester of pregnancy (OR 1.08 [95% CI 1.04, 1.12] per interquartile increase in CO concentration).[65]

Particulate matter

Exposure to fine and coarse particulate matter, $PM_{2.5}$ and PM_{10}, respectively, has been linked with fetal growth retardation. PM_{10} is emitted from residential heating and power plants, while $PM_{2.5}$ is emitted from cars, utility, or wood burning. Early fetal exposure to particulate matter can lead to altered trophoblast formation and inappropriate vascularization of the placenta.[74] In a

population based study, Dejmek et al. evaluated the impact of exposure to elevated levels of $PM_{2.5}$ and PM_{10} on risk of IUGR in a highly polluted area of Teplice, Northern Bohemia.[75] The risk for IUGR in relation to PM_{10} exposure during the first month of gestation was elevated for medium and high concentration levels of PM_{10} (OR 1.62 [95% CI 1.07, 2.50] and OR 2.64 [95% CI 1.48, 4.71], respectively). For $PM_{2.5}$, the OR was increased in the highest tertile of exposure (OR 2.11 [95% CI 1.20, 3.70]). Another study conducted in the same cohort reported an elevated risk for IUGR (OR 1.19 [95% CI 1.06, 1.33]) with each 10 $\mu g/m^3$ increase in PM_{10} concentration during the first month of pregnancy.[76] A recent study prospectively evaluated the impact of maternal exposure to air pollutants on fetal growth using ultrasound measurements as a direct estimate of growth.[77] Exposure to PM_{10} was significantly associated with SGA (OR 1.38 [95% CI 1.00, 1.90]).

Additionally, Mannes et al. assessed the relation between SGA and exposure to average levels of particulate matter during pregnancy among births reported in metropolitan Sydney from 1998-2000.[70] Exposure to $PM_{2.5}$ during the second trimester slightly elevated the risk of SGA birth (OR 1.03 [95% CI 1.01, 1.05]), whereas PM_{10} exposure was not significantly associated with SGA (OR 1.01 [95% CI 1.00, 1.04]). Parker et al. noted similar observations in a California birth cohort of 18,247 infants born to women exposed to $PM_{2.5}$ during pregnancy.[73] The odds of SGA with exposure to the highest quartile (>18.4 $\mu g/m^3$) of $PM_{2.5}$ was 1.23 [95% CI 1.03, 1.50]. In contrast, other studies have

reported null associations between maternal exposure to PM_{10} or $PM_{2.5}$ and SGA.[68, 72, 78]

Polycyclic aromatic hydrocarbons (PAHs)

PAHs are ubiquitous air pollutants generated by incomplete combustion of fossil fuels. They are human carcinogens and mutagens, and potential developmental toxicants.[76, 79] PAHs are readily absorbed on the surface of particulate matter forming DNA adducts that have been associated with reduced gestational length and decreased birthweight.[63, 79]

The association between PAH exposure and IUGR is unclear; although, some studies have reported positive findings. Dejmek et al. assessed the impact of PAH exposure on IUGR in Teplice and Prachatice regions of Czech Republic.[76] A significant increased risk was observed for IUGR (OR 1.22 [95% CI 1.07, 1.39]) with each 10 ng increase of PAH concentrations in air. Another study conducted in Poland reported that infants who had higher than median levels of PAH DNA adducts were more likely to have decreased birthweight, length, and head circumference.[79] Choi et al. also noted that prenatal exposure to PAH was significantly associated with reduced birthweight ($p < 0.01$) in two different cohorts, Krakow Caucasians and NYC African Americans.[80] A follow up study in the NYC cohort observed a 2 fold increase in risk of IUGR per natural log unit increase in PAH exposure among full term African American infants.[81]

The aforementioned studies that examined the relation between different environmental pollutants and SGA had several limitations. First, different measurement metrics were used for exposure assessment. Second, most studies failed to account for residential mobility; and third, average estimates of the pollutants were used for a given period that may have attenuated the results. Furthermore, exposure to air pollution is fairly common and majority of the air pollutants are highly correlated. Hence misclassification of exposure may have occurred.

The etiology and pathophysiology of SGA is not well established. Nitric oxide produced endogenously or formed from conversion of ingested nitrates and nitrites might play a role in the etiology of fetal growth restriction. Inoue et al. observed that an increase in serum nitrate/nitrite concentrations following administration of diethylenetriamine-nitric oxide significantly decreased placental and fetal weight in pregnant rats.[82] Studies have detected elevated nitric oxide levels in placenta and umbilical cord blood of pregnancies with intrauterine growth restriction compared to normal pregnancies.[83, 84] Further, another study noted that maternal and fetal nitrate and nitrite concentrations were significantly lower among women with SGA infants than those with appropriate for gestational age births implying that nitric oxide synthesis may be decreased in pregnancies with SGA infants.[85]

Certain medications, classified as containing nitrosatable amines (secondary or tertiary amines) or amides are precursors to the formation of N-

nitroso compounds. These compounds can be formed *in vivo* when nitrosatable amines or amides react with nitrosating agents such as nitrite in the stomach.[24] Endogenous formation of these compounds contributes to 40-75% of human exposure; however, exposure may also occur from exogenous sources, such as processed meat products, cured meats, smoked fish, alcohol, and tobacco.[86-88] Studies have shown N-nitroso compounds to cause congenital malformations in animal models but their effect on fetal growth is not known. Some drugs classified as tertiary amines form n-nitrosodimethylamine (NDMA) in the presence of nitrite.[89] An experimental study conducted in mice noted that acetoxymethyl-methylnitrosamine, which has the same active intermediate metabolite as NDMA, was associated with an increase in the number of weight-retarded fetuses.[90] Annola et al. detected transplacental transfer of NDMA in perfused human placentas from women who recently delivered full-term babies indicating that maternal exposure to NDMA may possibly affect fetal health.[91] Furthermore, in pregnant mice exposed to both ethylenethiourea (a nitrosatable compound) and nitrite, fetal weight was significantly reduced when both compounds were administered together, but no effect was observed when given separately.[92] These findings suggest that N-nitroso compounds, formed from combination of nitrosatable compound and nitrite, might influence fetal growth.

Numerous epidemiologic studies have examined the relation between maternal drug use during pregnancy and birthweight or SGA; but none of the studies evaluated the risk of SGA in relation to drugs classified as nitrosatable.

Only one published study examined the effect of nitrosatable drug exposure on fetal weight. Olshan and Faustman examined the relation between nitrosatable drugs use during the first four months and anytime during pregnancy and adverse pregnancy outcomes.[93] A significantly reduced risk was observed for low birthweight (<2000 grams) in relation to nitrosatable drug exposure during the given time periods.

A detailed review of drugs categorized by indication and classified as secondary or tertiary amines or amides and its association with SGA is covered below.

Secondary amines

Antidepressants

Depression is a common disorder among women of childbearing age with an estimated prevalence of 10 to 20%.[94] There has been a significant increase in prescription of antidepressants to women during pregnancy. Approximately 4 to 10% of women reported taking antidepressants during pregnancy, selective serotonin reuptake inhibitors (SSRI) was the commonly used antidepressant.[94] Although antidepressants reduce complications associated with depression; the safety of antidepressant use during pregnancy remains uncertain.

Several studies have reported positive associations between prenatal use of antidepressants and adverse pregnancy outcomes. Using data from the Slone Epidemiology Center Birth Defects Study, Toh et al. reported 7.2% of women exposed to antidepressants, specifically SSRI, anytime during pregnancy to

have increased risk (OR 1.7 [95% CI 1.0, 2.7]) of delivering an SGA offspring.[95] In addition, the risk for SGA was higher (OR 3.0 [95% CI 1.7, 5.5]) among those who continued use beyond the first trimester. Malm et al. observed similar observations in a population based study in which maternal SSRI use during pregnancy was compared between different trimesters and its effect on pregnancy outcome was evaluated.[96] Women who purchased SSRI during the second and third trimester (n=360) had increased risk for SGA births (OR 2.4 [95% CI 1.1, 5.3]), whereas exposure to SSRI during the first trimester or periconceptional period (n= 1,010) was not significantly associated with SGA (OR 1.9 [95% CI 1.0, 3.8]).

In a prospective cohort study, Chamber et al. evaluated pregnancy outcomes among women exposed to fluoxetine, an SSRI drug, during pregnancy.[97] Using the California Teratogen Information Service and Clinical Research Program, 228 pregnant women that received information on the teratogenic effects of fluoxetine and reported taking the drug were identified to be exposed and those who called regarding other nonteratogenic drugs (n= 254) were considered to be not exposed. The mean birthweight was significantly lower among those exposed to fluoxetine during late gestation (third trimester) compared to those exposed early or not exposed. In addition, the proportion of full term infants with birthweight at or below the 10 percentile were higher among those exposed later during pregnancy. Conversely, Wen et al. observed the risk for low birthweight to be elevated (OR 1.58 [95% CI 1.19, 2.11]) among women

who received an SSRI prescription a year prior to delivery compared to those who were not prescribed an SSRI.[98]

Colvin et al. also confirmed positive associations between exposure to SSRI during pregnancy and low birthweight.[99] The study linked data from population based health datasets in Western Australia and a national pharmaceutical claims dataset from 2002-2005. Approximately 3.8 percent of 96,968 pregnant women were dispensed a SSRI during pregnancy. Women dispensed an SSRI anytime during pregnancy were more likely to have low birthweight infant (OR 1.4 [95% CI 1.3, 1.6]) and the associations remained significant after controlling for other factors. The risk for low birthweight was also higher among women prescribed selective SSRI drugs including fluoxetine (OR 1.53 [95% CI 1.10, 2.14]), paroxetine (OR 1.44 [95% CI 1.12, 1.86]), and citalopram (OR 1.58 [95% CI 1.31, 1.91]). Furthermore, Kallen noted an increased risk for low birthweight among women who reported any antidepressant and SSRI use during pregnancy (OR 1.98 [95% CI 1.55, 2.52] and OR 1.98 [95% CI 1.42, 2.76], respectively).[100] However, no elevated risk was observed for SGA with any antidepressant or SSRI use (OR 0.83 [95% CI 0.53, 1.30] and OR 0.80 [95% CI 0.44, 1.44]).

In contrast, other studies found no statistically significant difference in birthweight or risk for SGA birth among women exposed to SSRI during pregnancy.[94, 101, 102] A recent Canadian study compared the incidence of SGA among women who contacted Motherrisk Program regarding antidepressant use

and those who requested information on other non-teratogenic drugs.[101] The risk for SGA was not significantly different among women exposed to antidepressants compared to controls (OR 1.19; 95% CI 0.86, 1.64). In addition, a retrospective cohort study of 228,876 singleton pregnancies among women enrolled in the Tennessee Medicaid Program, found no significant association between women receiving antidepressants during pregnancy and birthweight.[94]

Although, several studies reported associations between maternal SSRI use during pregnancy and increased incidence of low birthweight or SGA; other studies failed to detect a significant association. Additionally, the increased risk observed may not be attributable to the treatment itself but rather the indication for treatment since maternal depression has been linked with adverse neonatal outcomes. However, two studies that controlled for maternal depression reporting conflicting results.[102, 103] Wisner et al. conducted a prospective observational study to determine whether SSRI treatment in pregnant women with major depressive disorder was associated with increased risk for adverse neonatal outcomes.[102] Neither the mean birthweight nor the proportion of infants with birthweight below the 10th percentile differed across the exposure groups. In contrast, using propensity score matching to control for maternal illness severity, Oberlander et al. reported increased incidence of birthweight below the 10th percentile among pregnant women with depression exposed to SSRI compared to non-exposed women.[103]

Asthma medication

Asthma is one of the most common, potentially serious chronic disease in women of reproductive age, and occurs in 3.7% to 8.4% of all pregnancies.[104] Maternal asthma during pregnancy has been linked with an increased risk of pregnancy complications such as reduced infant size, intrauterine growth restriction, and prematurity as a result of poorly controlled asthma or some of the asthma medications used for treatment.[105] β_2 adrenergic agonists such as Albuterol are commonly used in management of asthma to relieve acute symptoms. Previous studies suggest that short acting β_2 agonists used for bronchodilation are generally considered safe during pregnancy. However, they are often used in conjunction with anti-inflammatory medications such as inhaled corticosteroids or leukotriene inhibitors that may increase the risk for adverse pregnancy outcomes.

In a prospective cohort study, Bakhireva et al. examined the effect of β_2 agonists on fetal growth among 654 infants born to women with asthma and 303 infants born to those without asthma.[106] The mean birthweight of full term infants was similar among women exposed to β_2 agonists (3552 g) and controls (3540 g). No significant difference was observed in the incidence of SGA births among the exposed group compared to non-asthmatic controls (OR 0.57 [95% CI 0.16, 2.12]). Similar findings were observed in a prospective cohort study conducted among members of the San Diego Kaiser Permanente Medical Care Program.[107] Pregnant women with asthma treated with bronchodilators (n= 259)

were identified and compared to 101 women with asthma and not exposed to bronchodilators. Risk of SGA birth was not significantly increased among pregnant women with asthma using inhaled bronchodilators compared to those with asthma and not using the drug or compared to controls. Bracken et al. also noted no significant increased risk for low birthweight among 401 pregnant women exposed to short acting β_2 agonists during the third trimester (OR 0.97 [95% CI 0.65, 1.47]).[108] Additionally, in a cohort of 2,123 asthmatic patients recruited from 16 centers of the National Institute Child Health and Human Development Maternal Fetal Medicines Unit Network, Schatz et al. confirmed null associations between maternal use of inhaled β_2 agonists and incidence of SGA infants.[109]

Data from the above reviewed studies indicate that management of asthma during pregnancy with β_2 agonists does not increase the risk of low birthweight or SGA birth.

Decongestants, pseudoephedrine

Decongestants are commonly used for the treatment of upper respiratory infections, allergy, and asthma during pregnancy. Approximately 22% of pregnant women reported rhinitis, and 25% visited the physician due to respiratory illness.[110] Decongestants are one of the most frequently used over the counter medications in pregnancy. Pseudoephedrine, the most commonly taken oral decongestant was reported to be used by at least 15% of women during pregnancy among 7,563 case and control mothers in the Slone

Epidemiology Center Birth Defects Study and 2,970 control mothers in the National Birth Defects Prevention Study.[111] While use of over the counter medications normally decreases during pregnancy, Werler et al. noted the frequency of pseudoephedrine use among pregnant women to increase from pre-pregnancy to the second trimester.[111]

Despite the frequent use of decongestants during pregnancy, few studies have evaluated the safety of decongestants during pregnancy and its effect on fetal growth. In a Swedish population based health study, Kallen & Olausson examined pregnancy outcomes among women who reported oral decongestant use during pregnancy.[110] During the study period (1995 – 2002), 2,474 women reported use of oral decongestant during early pregnancy, and 1,771 women used prescription decongestants later in pregnancy. Women who reported use of decongestants during the first trimester had increased risk of SGA births (OR 1.07 [95% CI 1.07, 1.42]), while use of decongestants during the second and third trimester was associated with a reduced risk of SGA (OR 0.71 [95% CI 0.47, 1.08]). The discordant results observed may be due to multiple testing or the indication of use, probably pregnancy rhinitis. Kallen & Olausson also studied concomitant drug use among those exposed to oral decongestants during pregnancy.[110] The majority of women exposed to oral decongestants also reported use of antihistamines and asthma medication during pregnancy which indicated the presence of an allergic rhinitis. Approximately 30% of women using oral decongestants during early pregnancy also used antibiotics or cough

medications indicating the possibility of an upper respiratory infection. Although conflicting results were observed with oral decongestants use, findings may be confounded by other concomitant drug use and indication.

Antidiabetic, biguanides

Metformin, an oral hypoglycemic agent, classified as secondary or tertiary amines, is commonly used in the management of gestational diabetes mellitus (GDM). The overall incidence of GDM is reported as 3-6% but has steadily increased over time and varied widely between the racial groups. Insulin is generally considered as the standard management for gestational diabetes mellitus, when diet and exercise fail to achieve normal glucose levels.[112] However, less compliance has been achieved with insulin therapy among pregnant women. Oral hypoglycemic agents are used as an alternative treatment option among women with GDM. Furthermore, the convenience of oral medication, simple dosages, and low costs increased their use during pregnancy. There is limited evidence on the safety of oral hypoglycemic agents, specifically metformin therapy.

Previous studies have compared pregnancy outcomes in women with GDM treated with metformin or insulin therapy.[113-115] In a prospective cohort study, Moore et al. randomly assigned pregnant women with GDM receiving prenatal care at University of Mississippi Medical Center to receive metformin or insulin therapy.[114] There was no statistically significant difference observed in birthweight of infants between the metformin and insulin group ($p < 0.806$).

Rowan et al. noted similar findings in a randomized open trial comparing metformin with insulin therapy among 751 pregnant women with GDM.[115] Women were randomly assigned to open treatment with metformin (with supplemental insulin if required) or insulin. Of 326 women assigned to metformin therapy, 92.6% continued metformin use until delivery. The proportion of infants with birthweight below the 10 percentile differed slightly between the two groups (7.2% v. 9.7%), however no statistically significant difference was observed. Furthermore, Hughes & Rowan reported birthweight to be similar among infants born to women with GDM treated with metformin and those not exposed to metformin.[113]

Findings from the above reviewed studies suggest that metformin therapy used for the management of GDM during pregnancy is not associated with increased risk of low birthweight or SGA infants.

Cardiovascular medications

Certain cardiovascular drug groups such as diuretics, beta blockers, calcium channel blockers, and antihypertensives classified as either secondary or tertiary amines or amides have presented conflicting results in relation to its association with SGA.

Diuretics, thiazide

Diuretics classified as secondary amines or amides are used for treatment of hypertension and cardiac diseases. The use of diuretics during

pregnancy has been associated with increased birthweight, possibly due to the diabetogenic side effects of these drugs.[116, 117] Olesen et al. observed conflicting results between the effect of thiazide and loop diuretics on birthweight.[118] Two retrospective cohort studies were conducted. Women who purchased prescription diuretics during pregnancy were identified from the Northern Jutland Prescription Database, Denmark, and the Medicines Monitoring Unit Database, Scotland. Danish women who purchased prescription loop diuretics during pregnancy had infants with higher birthweight, mean difference of 105 grams [95% CI 2.6, 206.9] compared to those who did not use diuretics. Those who purchased thiazide diuretics during pregnancy were at higher risk of low birthweight infants (OR 2.6 [95% CI 1.4, 5.0]). The risk for low birthweight was also elevated among Scottish women exposed to thiazide diuretics during pregnancy (OR 2.4; 95% CI 0.8, 7.8). However, higher prevalence of diabetes (10.3%) among Danish women who purchased loop diuretics might explain the discordant results observed between thiazide and loop diuretics. Furthermore, the prevalence of hypertension was higher (15.8%) among women who purchased thiazide diuretics. Thus, the increased risk observed for low birthweight may be linked to indication for prescription rather than the treatment itself.

Beta blockers

Beta blockers, classified as secondary or tertiary amines or amides according to nitrosatability, are widely used in the treatment of chronic

hypertension, migraine, heart failure, tremors, and other conditions. Previous studies have reported positive associations between β blocker treatment during pregnancy and SGA.

In a population based study, Petersen et al. investigated the associations between β blocker exposure during pregnancy and risk of being born SGA in a Danish birth cohort, comprising 974,805 births between 1995 and 2008.[119] Redeeming prescriptions of β blockers during pregnancy was found to be significantly associated with increased risk of SGA birth (OR 1.97 [95% CI 1.75, 2.23]). Specifically, labetalol, a β blocker considered to be safe during pregnancy was significantly associated with SGA (OR 2.02 [95% CI 1.72, 2.37]). Exposure to other β blockers (metoprolol, atenolol, propranolol, pindolol, and sotalol) also increased the risk of delivering a SGA infant (OR 2.01 [95% CI 1.66, 2.43]). Using a population health dataset in Taiwan, Ho et al. evaluated the effect of antiglaucoma medications during pregnancy on risk of having low birthweight infants.[120] Mothers prescribed topical β blockers for glaucoma during pregnancy had an elevated risk of having a low birthweight infant (OR 1.48 [95% CI 0.86, 2.56]) compared to those not taking these drugs.

Several studies examined the effect of timing of atenolol (β blocker) exposure on fetal growth.[121-123] Atenolol is a selective β_1 blocker primarily used in the treatment of hypertension and angina or chest pain. Using data from the antenatal hypertensive pregnancy database of two district hospitals in England, Bayliss et al. evaluated the effect of atenolol exposure during early pregnancy

on birthweight. Infants born to women taking atenolol during the first trimester had significantly lower birthweight compared to those not taking any medication ($p < 0.01$).[121] Additionally, the risk for having SGA infants was higher among those exposed to atenolol < 15 weeks of gestation (OR 2.81 [95% 1.27, 6.24]). The birthweight of infants was also significantly lower among women taking atenolol, labetalol, or methyldopa drugs during the second trimester of pregnancy. Similar findings were noted by Lip et al. who found mean birthweight to be significantly lower among women taking atenolol during pregnancy compared to controls ($p < 0.001$).[122]

Lydakis et al. investigated the effect of duration of atenolol monotherapy in a retrospective cohort study of 312 pregnancies in 223 women attending an Antenatal Hypertension Clinic.[123] Atenolol was given in 78 (25%) pregnancies and 91 pregnancies (29.2%) received no antihypertensive drugs. Women who received atenolol in early pregnancy (<20 weeks) had significantly lighter and smaller babies, when compared to those exposed to atenolol later during pregnancy (>30 weeks). There was a significantly higher proportion of SGA babies (70%) in the group with early onset of treatment (<20 weeks).

With increasing use of β blockers during pregnancy, specifically atenolol and other β blockers (labetalol, propranalol, metoprolol) prescribed for hypertension and cardiac disease management, the safety of these drugs during pregnancy need to be examined. Although the aforementioned studies found positive associations between various β blockers and SGA, especially with

exposure during early pregnancy, findings need to be corroborated by future studies.

Gastrointestinal H2 blocker

Pregnancy is commonly complicated by gastroesophageal reflux disease with this condition affecting 30% to 50% of pregnant women.[124] H_2 receptor antagonists such as ranitidine, famotidine, and cimetidine inhibit gastric secretion and generally used for the treatment of peptic ulcer and acid reflux disease. They are classified as secondary or tertiary amines according to nitrosatability. Although these drugs are known to cross the placenta by passive diffusion, animal toxicological studies have failed to show teratogenic effects. Published literature regarding the safety of intrauterine exposure to H_2 blockers is limited, although most studies reported null associations with low birthweight or SGA.

Using data from the European Network of Teratology Information Services, Garbis et al., in a prospective cohort study, evaluated pregnancy outcomes in 553 pregnancies exposed to H_2 blockers during pregnancy.[125] The majority of women (n = 501) reported use of H_2 blockers during the first trimester, while 51 women reported usage during the second or third trimester. Mean birthweight of infants born at term was not significantly different between the exposed and control group ($p < 0.850$). In a large hospital cohort, Matok et al. investigated the safety of H_2 blockers during pregnancy among members of Clalit Health Services in Israel from 1998 to 2007.[126] Exposure to H_2 blockers

was evaluated by each trimester, and 1,148 women were dispensed these drugs during the first trimester. Use of H_2 blockers during first, second, and third trimester was not associated with increased risk of low birthweight infant.

Similar findings were noted by Magee et al. in a prospective cohort study conducted at the Motherrisk Program, Canada.[127] Women who contacted about gestational exposure to H_2 blockers were identified as exposed and those who enquired about non-teratogenic drugs as controls. H_2 blockers were most often used during the first trimester (88%) and 13% reported use throughout pregnancy. No difference was observed between the two groups for incidence of SGA (p 0.19). Furthermore, Ruigomez et al. reported null association between first trimester exposure to H_2 blockers (cimetidine, ranitidine, and omeprazole) and SGA in two cohorts identified from the United Kingdom general practice research database and the Italian Friuli-Venezia Giulia health database.[128] Previous studies found no increased risk for SGA with exposure to H_2 blockers during pregnancy. H_2 blockers may be considered safe to be used during pregnancy, however a possible teratogenic risk cannot be ruled out.

Migraine

Migraine headaches affect 16% to 21% of the general population.[129] It is more frequently seen in fertile women and attacks can occur in relation to menstruation or ovulation. In pregnancy, the frequency of migraine tends to decrease throughout gestation, especially during the second and third trimesters.[130] Triptans, classified as secondary or tertiary amines or amides, are

derivatives of tryptamines, and act as serotonin agonists by binding to its various receptors. Triptans are generally considered safe during pregnancy except drugs such as ergotamine and dihydroergotamine are contradicted during pregnancy. Studies concerning the possible adverse effects of triptans, most notably sumatriptans, first class of drugs of triptans have yielded conflicting results.

Using data from the Swedish Medical Register, Kallen & Lygner evaluated delivery outcomes among women using drugs for migraine during pregnancy.[131] Approximately 912 women reported use of drugs indicated for migraine during pregnancy, and majority of them reported use of sumatriptan (n = 658). Risk for low birthweight was slightly elevated among mothers reporting use of drugs for migraine (OR 1.04 [95% CI 0.67, 1.61]), and the risk was most notable with sumatriptan exposure (OR 1.18 [95% CI 0.70, 1.97]) although the 95% CI did not reach statistical significance. Olesen et al. noted similar observations between sumatriptan exposure during pregnancy and low birthweight.[132] Linking data from the Pharmaco-Epidemiological Prescription Database of North Jutland and the Danish Medical Registry, 34 pregnant women exposed to sumatriptan were identified and compared to 89 control individuals with migraine not using the drug and 15,955 healthy women. The odds of having an infant with low birthweight was increased (OR 2.3 [95% CI 0.3, 17.6]) among migraine patients receiving sumatriptan treatment compared to healthy controls. However, lower risk was noted when migraine patients were used as controls

(OR 0.9 [95% CI 0.1, 11.8]). Findings observed may be confounded by the drug indication and severity of the disease.

In contrast, Kallen et al. reported negative associations between first trimester exposure to drug prescribed for migraine and SGA.[133] In a large cohort study of 1,211,670 women, use of triptans or ergots during the first trimester was reported by 3,286 women, while use after first trimester occurred in 1,394 women. Women exposed to migraine drugs during the first trimester had lower risk for delivering an SGA infant (OR 0.95 [95% CI 0.75, 1.20]). An increased risk for SGA was also observed with exposure during the second and/or third trimester (OR 1.20 [95% CI 0.86, 1.68]) although the confidence intervals were not significant. However, the positive associations observed may be due to other drug groups notably ergots and also confounded by the maternal disease.

Tertiary amines

Antiepileptics

Epilepsy is a common neurologic disorder in pregnant women with a prevalence of 0.3-0.7%.[134] Epilepsy is considered a risk factor for several pregnancy outcomes like preeclampsia, placental bleeding, and preterm birth. However, it is unclear whether the complications are due to epilepsy or the use of antiepileptic drugs.

Studies have reported conflicting results with regard to antiepileptic drug use and SGA. In a retrospective cohort study, Hvas et al. investigated birth outcomes among pregnant women who received prenatal care at Aarhus

University Hospital, Denmark from 1989 to 1997.[135] The mean birthweight was observed to be reduced by 208 g [95% CI 116, 300] among women with epilepsy receiving anticonvulsant treatment compared to women without epilepsy. The reduction was most pronounced among children of women receiving carbamazepine and oxcarbazepine monotherapy. The risk for SGA was increased (OR 2.3 [95% CI 1.3, 4.0]) among those who received antiepileptic therapy compared to controls. Fonager et al. also reported mean birthweight to be 46 g lower [95% CI -110, 18] among women exposed to anticonvulsants around conception and during pregnancy compared to those not exposed.[136] The risk for low birthweight was higher among those exposed to anticonvulsants compared to controls (OR 1.5 [95% CI 0.6, 3.7]). In addition, a meta-analysis study noted birthweight to be reduced in children of women who received antiepileptic treatment during pregnancy.[137]

Using data from the Medical Birth Registry of Norway, Veiby et al. investigated delivery outcomes in a large cohort of 2,861 women with epilepsy and compared to 369,267 women without epilepsy from 1999-2005.[138] The majority of epileptic women (66%, n= 1900) did not report use of antiepileptic drugs while 961 women reported use during pregnancy. Risk of SGA was significantly increased among women exposed to antiepileptic drugs compared to those not exposed (OR 2.0 [95% CI 1.4, 2.7]). Exposure to carbamazepine during pregnancy also elevated the risk for delivering an SGA offspring (OR 2.7 [95% CI 1.7, 4.0]). Similar findings were noted by Viinikainen et al. in a

retrospective cohort study of 24,778 infants born from 1989-2000 at Kuopio University Hospital, Finland.[139] Exposure to monotherapy with carbamazepine revealed a trend towards SGA infants, but the difference was not statistically significant.

Conversely, other studies reported no difference in mean birthweight and no increased risk was observed for SGA among epileptic women who received antiepileptic drugs compared to those without epilepsy.[140, 141] Katz et al. retrospectively analyzed 100 consecutive pregnancies from 1990-2000 among women with epilepsy.[140] The mean birthweight of infants was observed to be similar among women exposed and not exposed to antiepileptic drugs during pregnancy. Using data from two national population databases, Lin et al. evaluated the risk of adverse pregnancy outcomes among epileptic women who received antiepileptic treatment.[141] No significant difference in the risk of SGA infant was observed between epileptic women who were given antiepileptic drug use and those without epilepsy (OR 1.45 [95% CI 0.98, 2.10]).

Overall, antiepileptic drug use during pregnancy increased the risk for reduced birthweight and SGA infants.[135-139] However, other studies did not find any significant difference.[140, 141] Findings may be confounded by severity of epilepsy and smoking status. Antiepileptic drugs may reduce the availability of folate and low serum folates have been associated with low birthweight.[135] Further evaluation of the effect of antiepileptic drug treatment on birthweight is warranted.

Opioids

Methadone, a synthetic opioid is generally used for the management of maternal opioid dependency during pregnancy. Several studies have shown intrauterine growth restriction to be a common feature in pregnancies of opioid dependent mothers. Wouldes & Woodward examined the association between maternal methadone dose during pregnancy and a range of infant outcomes.[142] Two groups of infants, methadone exposed (n = 32) and non-exposed (n =42) born at the National Women's Hospital, New Zealand were followed longitudinally from the third trimester of pregnancy to 6-7 months of age. Significant linear associations were found between maternal methadone exposure and birthweight ($p < 0.01$), especially with higher dose of methadone. Infants born to women exposed to high dose of methadone treatment (>58 mg/day) were more likely to have a SGA infant ($p < 0.05$). In addition, maternal methadone remained a significant predictor of birthweight after adjusting for maternal and infant confounding factors. Liu et al. also reported methadone treatment in opioid dependent mothers to be associated with birthweight. In a retrospective cohort study, neonatal parameters from pregnancies of opioid dependent mothers maintained on methadone treatment were compared to non-smoking opioid dependent mothers.[143] Infants born to opioid dependent mothers given methadone treatment had significantly lower birthweight compared to controls ($p < 0.001$). Additionally, the number of infants with birthweight below the 10 percentile was significantly higher in the exposed group compared to

controls (27% v. 9%). Cleary et al. confirmed findings from the previous studies.[144] A retrospective cohort study of 61,030 singleton births was conducted at a large maternity hospital from 2000-2007. Approximately 618 (1%) women received methadone at delivery. Methadone exposure was associated with an increased risk of being born small for gestational age (OR 2.21 [95% CI 1.85, 2.64]) with adjustment for maternal risk factors.

Methadone treatment among opioid dependent mothers significantly increased the risk for low birthweight or SGA. However, the beneficial effects of minimizing illicit drug use and maintaining the pregnancy may outweigh the risks of neonatal outcomes associated with methadone treatment.

Antihypertensives

Hypertension during pregnancy increases the risk of perinatal mortality and morbidity. Antihypertensive drugs may help reduce maternal morbidity from the complications of high blood pressure and prolong the pregnancy. However, studies have hypothesized that reduction in mean arterial blood pressure could decrease placental perfusion and diminish fetal growth. The following drug groups are used for control of hypertension: beta blockers, alpha adrenergic blockers, angiotensin converting enzyme inhibitors, calcium channel blockers, angiotensin receptor 2 antagonists, central acting adrenergic, and diuretics. Associations observed with beta blockers, specifically atenolol were discussed earlier.

Two studies examined the effect of overall antihypertensive drug use on birth outcomes.[145, 146] Using data from Swedish Medical Birth Register, Lennestal et al. investigated the association between antihypertensive drug use and delivery outcomes.[145] Approximately 1,418 women reported antihypertensive drug use during early pregnancy and majority of them (84%) used drug from one category. While 14% reported use of drugs from two categories and 1.3% used drugs from three or four different groups of antihypertensives. Overall, antihypertensive drug use was associated with a higher risk for SGA births (OR 4.23 [95% CI 3.55, 5.03]). The risk for having low birthweight infants was also elevated (OR 4.72 [95% CI 4.11, 5.41]). Nakhai-Pour et al. noted increased risk of SGA in relation to use of antihypertensives later during pregnancy.[146] Using data from the Quebec Pregnancy Registry, drug exposure was compared between 7,445 babies born SGA and 48,889 control babies. Adjusting for potential confounders, antihypertensive drug use during the second or third trimester was associated with a 53% increased risk for SGA compared to those not exposed (OR 1.53 [95% CI 1.17, 1.99]). Risk of SGA births was also elevated with selective beta blockers, non-selective beta blockers, and central adrenergic drug use during the second or third trimester of pregnancy. No significant association was observed between antihypertensive use during the first trimester and SGA (OR 1.07 [95% CI 0.69, 1.97]).

Although the aforementioned studies supported an association between overall antihypertensive drug use and SGA, the effect of dosage and duration of antihypertensive drug use needs to be determined.

Calcium channel blocker

Calcium channel blockers are classified as tertiary amines or amides based on the nitrosatable groups present in their molecular structure. Magee et al. prospectively collected information from six teratogen databases and followed 78 women who were exposed to calcium channel blockers during the first trimester.[147] A trend towards decreased birthweight was observed among those exposed compared to controls (3018 g v. 3352 g, p = 0.08). The proportion of small for gestational age infants was higher in the treatment group compared to controls (6.6% v. 1.5%), although the difference was not statistically significant (p = 0.13).

Antiinfective, macrolide

Macrolides classified as tertiary amines or amides, act by inhibiting bacterial protein biosynthesis. They are primarily used in the treatment of respiratory infection, bacterial skin infection, and chlamydia infection. In pregnancy, they are used for the treatment of endocervical chlamydial infections produced by Mycoplasma pneumonia and by group B streptococcus and among women who are allergic to B lactam antibiotics.[148] The most commonly used macrolides include erythromycin, azithromycin, clarithromycin, roxithromycin,

and spiramycin. Only a handful of studies have studied the relation between exposure to macrolides during pregnancy and low birthweight or SGA.

Kallen et al. investigated delivery outcomes among women exposed to erythromycin during early pregnancy based on data obtained from the Swedish Medical Register.[149] Approximately 1,844 women reported exposure to erythromycin before, and 1,831 after the first antenatal visit. No statistically significant risk was observed for SGA among infants born to women exposed to erythromycin during early pregnancy (OR 0.92 [95% CI 0.65, 1.30]). In a large population cohort of 180,120 pregnant women, Romoren et al. compared birth outcomes between women who took erythromycin, penicillin V, or amoxicillin in the first trimester of pregnancy and those who were not exposed to any systemic antibiotics during the same period.[150] Among 5,729 women who reported erythromycin use anytime during pregnancy, the risk for low birthweight was lower among those exposed compared to controls (OR 0.85 [95% CI 0.68, 1.05]). Similar findings were noted by Andrews et al. in a randomized control trial evaluating the efficacy of treatment with erythromycin plus metronidazole.[151] Women assigned to the treatment group had lower risk of having low birthweight infants compared to those in the placebo group (OR 0.88 [95% CI 0.60, 1.29]). In addition, Chun et al. reported no difference in birthweight among pregnant women exposed to roxithromycin during early pregnancy compared to controls.[148]

Antiemetics

More than half of pregnant women suffer from nausea and vomiting during pregnancy, primarily during the first trimester.[152] The condition occurs at an increased rate in young women, in multiparous women, and twin pregnancies. Antiemetics, classified as tertiary amine or amide are prescribed to control the severity of nausea and vomiting until it subsides. Oral antihistamines (diphenhydramine, promethazine, meclizine, cyclizine), dopamine modulators (metoclopramide), and serotonin 5HT3 receptor antagonists (ondansetron) are antiemetics available for the treatment of nausea and vomiting during pregnancy.

Asker et al. examined neonatal outcomes among 676,198 births registered with the Swedish Medical Birth Registry from July 1, 1995 to December 31, 2002.[152] During this period, 29,804 (4.5%) pregnant women reported use of antiemetic drugs, 86% of whom reported use before the first antenatal visit. Meclozine was the most frequently taken antiemetic drug (68%) and promethazine was the second most commonly reported drug. A lower risk was observed for low birthweight and SGA infants with any antiemetic drug use during pregnancy (OR 0.89 [95% CI 0.83, 0.96] and OR 0.90 [95% CI 0.82, 0.99]). However, a slightly elevated risk was observed for low birthweight with promethazine use during pregnancy (OR 1.21 [95% CI 1.06, 1.39]). The better infant outcome observed with any antiemetic use may not be due to a direct drug effect but probably a well-functioning placenta. Placental hormones are

proposed to play a role in the etiology of nausea and vomiting and better placenta function increasing the probability of a good pregnancy outcome.

Diav-Citrin et al. compared pregnancy outcomes among women exposed to loratadine, and other oral antihistamines during pregnancy.[153] Women who contacted the Israeli Teratogen Information Service between 1995 and 2001 with regard to information about loratadine, other oral antihistamines, and non teratogenic drugs were prospectively identified and followed. Approximately 210 (77.9%) pregnancies were exposed to loratadine and 267 (64.6%) to oral antihistamines during the first trimester, and compared to 929 non teratogenic controls. The mean birthweight did not significantly differ between the three groups ($p = 0.302$).

In the United States, metoclopramide is only used in severe cases of nausea and vomiting. While in some European countries and Israel metoclopramide is a commonly used antiemetic. In a retrospective cohort study, Matok et al. investigated the safety of metoclopramide use during the first trimester of pregnancy among 81,703 infants born to women registered with the Clalit Health Services, Israel.[154] Exposure to metoclopramide was not associated with increased risk of low birthweight (OR 1.01 [95% CI 0.89, 1.14]) compared to those not exposed to the drug.

The combination of doxylamine succinate (antihistamine) and pyridoxine (vitamin B6) was the most widely used preparation for treatment of nausea and vomiting in the 1960-1970s. However, due to litigations and despite evidence of

fetal safety, the drug was voluntarily removed from the market of United States in 1983. The drug continued to be used in Canada for treatment of the condition. Atanackovic et al. examined pregnancy outcomes in 225 women who took the drug combination at the recommended (n = 123) or higher than recommended (n = 102) doses.[155] Birthweight was not associated with the drug dose and duration of treatment.

Nicotine replacement therapy

Maternal smoking during pregnancy is a known risk factor for fetal growth restriction and other adverse pregnancy outcomes. Nicotine replacement therapy (NRT), classified as tertiary amines, has been shown to be an effective smoking cessation therapy among non-pregnant smokers. However, its efficacy and safety during pregnancy and effect on fetal growth is not known.

Lassen et al. examined the relation between NRT use during pregnancy and birthweight in offspring of 72,761 women enrolled in the Danish National Birth Cohort.[156] No significant association was observed between the duration of NRT use and birthweight (β = 0.25 g [95% CI -2.31, 2.81], average change in birthweight with an increase of NRT use by 1 week). However, simultaneous use of more than one NRT product was associated with reduced birthweight (β = -10.73 g [95% CI -26.51, 5.05]), although the association was not significant. In contrast, using data from the 2004 Phase V Pregnancy Risk Assessment Monitoring System, Gaither et al. reported twice the risk of low birthweight (OR 1.95 [95% CI 1.10, 3.46]) among women who were recommended NRT

compared to non-smokers.[157] Although, the women prescribed NRT reported higher frequency of smoking and used NRT inconsistently.

A systematic review of two studies that evaluated pregnancy and fetal outcomes in women using NRT during pregnancy found no significant difference in mean birthweight of infants between the NRT and placebo groups.[158] However, only one study that evaluated effects of nicotine gum reported higher mean birthweight and a decreased risk for low birthweight ($p < 0.001$) among women in the NRT group.[159] Furthermore, another meta-analysis study noted the pooled estimates for mean birthweight to be significantly higher among infants born to women in the NRT group.[160] A mean difference of 158.2 g [95% CI -53.13, 369.52] with high level of heterogeneity ($I^2 = 76\%$) was observed.

Findings from the previous studies suggested that NRT use during pregnancy may be not associated with low birthweight. Future studies should examine the dose, timing and duration of NRT use on infant weight. Since smoking is harmful to the fetus, NRT may be an effective alternative for smoking cessation.

Amides

Antiinfective, beta lactam

Beta lactam antibiotics, classified as amides, represent the oldest class of antibiotics used for the treatment of infections. They are bactericidal, and act through the inhibition of bacterial cell wall synthesis. Amoxicillin, a *β* lactam antibiotic is used for the treatment of various infections such as tonsillitis,

pneumonia, otitis sinusitis, and urinary tract infections.[161] It was among the 20 most commonly reported prescription medication during the first trimester of pregnancy among the NBDPS participants from 1997-2003.[162] While several studies have evaluated the effect of β lactam antibiotics on pregnancy outcomes, limited data is available on the safety of amoxicillin use during pregnancy.

Using data from Danish population based registries, Jespen et al. examined the association between amoxicillin exposure during pregnancy and birth outcomes.[163] A cohort of 401 primiparous women who redeemed a prescription for amoxicillin during pregnancy was compared to 10,237 women who did not redeem any prescription from three months before until the end of pregnancy. The mean birthweight of infants born to amoxicillin exposed mothers was 57 g [95% CI 9, 105] higher than compared to controls. The odds of having a low birthweight infant was also lower among mother exposed to amoxicillin compared to controls (OR 0.63 [95% CI 0.26, 1.53]). Two other studies that used the same Danish medical and prescription databases reported null associations between pivampicillin or pivmecillinam exposure, drugs closely related to amoxicillin, and low birthweight.[164, 165] Larsen et al. reported the mean birthweight to be 35 g higher among women to pivampicillin compared to controls.[164] No increased risk was observed for low birthweight (OR 0.93 [95% CI 0.55, 1.57]) with this drug exposure. Skriver et al. also noted no significant

risk for low birthweight among 2,031 pregnant women exposed to pivmecillinam during pregnancy (OR 0.79 [95% CI 0.52, 1.20]).[165]

Because of the increasing resistance of bacteria to β lactam antibiotics, amoxicillin is prescribed in combination with a β lactamase inhibitor such as clavulanic acid. Berkovitch et al. evaluated the safety of combination of amoxicillin and clavulanic acid in 191 women recruited from the teratogen information services in Israel.[161] Pregnancy outcomes were compared between women exposed to the amoxicillin/clavulanic acid combination and amoxicillin only. No significant difference in birthweight was observed between the two groups.

Antiinfective, sulfonamide

Sulfonamides are used in the treatment of variety of infections, and are commonly used for the treatment of acute and uncomplicated urinary tract infections in women. Sulfonamides cross the placental barrier, and achieve blood levels in fetus as high as 90% of those in the maternal circulation.[166] Studies have reported inconsistent results between maternal exposure to sulfonamides and SGA or low birthweight.

In a retrospective cohort study, Yang et al. evaluated birth outcomes in a 50% random sample of women who gave birth in the Canadian province of Saskatchewan from 1997-2000.[167] Approximately 447 women reported exposure to trimethoprim/ sulfamethoxazole during pregnancy. A significantly increased risk (OR 1.67, 95% CI 1.14, 2.46) for low birthweight was observed among the

exposed compared to controls. Using the Quebec Pregnancy Registry, Santos et al. examined the association between antiinfective drug exposure during the second and third trimester of pregnancy and risk of SGA births.[168] No increased risk was observed for SGA with exposure to all combined antiinfective drugs (OR 0.97 [95% CI 0.91, 1.04]). However, the use of sulfamethoxazole during the second or third trimester of pregnancy was significantly associated with SGA (OR 1.61 [95% CI 1.16, 2.23]). In contrast, Ratanjamit et al. reported a reduced risk for low birthweight (OR 0.69 [95% CI 0.49, 0.98]) among women prescribed sulfamethizole 30 days before conception to the date of delivery compared to those not exposed during the same period.[166]

Benzodiazepine

Benzodiazepines, classified as amide or tertiary amines, are class of drugs primarily used for treating anxiety, but they are also effective in treating seizures and insomnia. These drugs cross the placenta and have the potential to accumulate in the fetus. Data on the effect of benzodiazepines with neonatal outcomes is sparse.

In a retrospective cohort study, birth outcomes were examined among 1,979 infants whose mothers reported use of benzodiazepines during early pregnancy, and 390 exposed later during pregnancy.[169] An increased risk for SGA was observed among infants exposed, both in early and late pregnancy (OR 1.12 [95%CI 0.87, 1.44] and OR 1.39 [95%CI 0.80, 2.40], respectively),

although the ORs were not statistically significant. The weak associations observed in this study need to be confirmed by future studies.

Dietary intake and SGA

Dietary nitrates and nitrites contribute a significant portion of daily nitrite exposure compared to other known sources of exposure (drug use and water consumption). Vegetables are main source of nitrates and cured meat, baked goods, and cereals are common sources of dietary nitrite.[170] Previous epidemiologic studies have examined the role of maternal dietary patterns;[8-10] nutrient intake;[14-16] and supplementation on fetal growth;[14, 15, 18] but none studied the effect of dietary nitrate/nitrites on fetal weight. In a cohort of 1,714 women, Thompson et al. evaluated the effect of different maternal dietary patterns during pregnancy on the risk of delivering an SGA infant.[9] Mothers who adopted a traditional diet during early pregnancy, that included mostly fruits and vegetables, were less likely to have an SGA infant (OR 0.86 [95% CI 0.75, 0.99]). Knudsen et al. also explored the association between maternal dietary patterns and fetal growth among 44,612 women in Denmark.[8] Two major dietary patterns, western and health conscious, were identified with the first pattern characterized by red and processed meat, and high fat dairy intake, while the second pattern included consumption of vegetables, fruits, poultry, and fish. Women with health conscious diet had lower risk of SGA (OR 0.74 [95% CI 0.64, 0.86]) compared to those in the western diet group. Furthermore, using data from the United States Hispanic Health and Nutrition Examination Survey,

Wolff et al. evaluated dietary patterns among Mexican American women during pregnancy.[10] Nutrient dense (fruits, vegetable, low fat dairy) and protein rich (low fat meat, processed meat, dairy desserts) dietary patterns were significantly associated with increased birthweight in offspring.

Individual macronutrients and total energy intake during pregnancy may also influence fetal growth. Moore et al. assessed the relation between maternal dietary composition during pregnancy and birthweight among 557 women in Adelaide, South Australia.[16] In early pregnancy, the proportion of energy derived from protein (17%) was positively associated with birthweight and placental weight. Each percent increase in protein was associated with a 16 g increase in birthweight [95%CI 2.8, 29.2] and 4.2g increase in placental weight [95% CI -0.4, 8.5]. Godfrey et al. reported similar findings among 538 infants delivered at term at a maternity hospital in Southampton, England.[14] Lower maternal intake of protein was associated with decreased birth parameters. Placental weight and birthweight decreased by 1.4g [95% CI 0.4, 2.4] and 3.1g [95% CI 0.3, 6.0], respectively, for each gram decrease in intake of dairy and meat protein during late pregnancy. In addition, mothers who reported high carbohydrate intake during early pregnancy had infants with lower placental and birthweight. Conversely, other studies noted null associations between maternal nutrient intake and birth size measures.[15, 171]

Vitamin C supplementation and SGA

Vitamin C is known to inhibit nitrosation. Mirvish et al. first demonstrated that ascorbic acid inhibits N-nitroso compound formation by rapid reduction of nitrite to nitrous oxide, followed by the production of dehydroascorbic acid.[172] In a clinical trial conducted with human volunteers, increased doses of ascorbic acid (1.76 -1000mg) with combined exposures to nitrate and proline (a nitrosatable precursor) significantly reduced the excretion of N-nitroso compounds by 44 percent compared to exposures to nitrate and proline without concomitant administration of vitamin C.[173] In our previous studies, we examined the effects of vitamin C supplementation on risk of selected birth defects among women exposed to nitrosatable drugs during pregnancy.[174, 175] Relative to women with no vitamin C supplementation, daily use of vitamin C supplement in conjunction with tertiary amine exposure during the periconceptional period was associated with lower odds of anencephalic births.[174] Lower ORs were observed for selected birth defects with daily vitamin C supplementation and nitrosatable drug use during the first trimester including transverse limb deficiency with secondary amines, cleft lip without cleft palate with tertiary amines, and several congenital heart defects with tertiary amines and amides.[175]

Very few studies have examined the association between maternal vitamin supplementation during pregnancy and risk of SGA birth.[15, 18, 176, 177] In a prospective cohort study, Alwan et al. noted no significant associations between use of multivitamins during pregnancy and SGA.[176] The ORs marginally

decreased from 1.3 ([95% CI 0.8, 1.9]) to 0.9 ([95% CI 0.5, 1.7]) with vitamin supplement use from the first to third trimester of pregnancy. Another study indicated that maternal use of vitamin supplement during the last month of pregnancy was associated with a slightly lower risk of SGA birth (OR 0.76 [95% CI 0.55, 1.05]).[18] Mathews et al. found total vitamin C (estimated from food and supplement) intake during early pregnancy to be positively associated with birthweight, with a mean difference of 100g noted between the lowest (< 55 mg/day) and highest thirds (≥ 98 mg/day) of intake.[15] Additionally, a systematic review study reported no significant difference in risk of SGA birth between the treatment group that received a combination of vitamin C and E compared to the placebo group (20.6% v. 20%, RR 0.94 [95% CI 0.74, 1.19]).[177] However, none of the studies assessed the effect of vitamin C supplement use on risk of SGA in relation to nitrosatable drugs use during pregnancy.

Methods

To examine the association between maternal dietary nitrate, nitrites, and nitrosatable drugs and risk of SGA births, data were used from the National Birth Defects Prevention Study (NBDPS) control mothers who delivered infants without major congenital malformations with estimated dates of delivery (EDD) from 1997 to 2005. The NBDPS, a large US population-based case control study of birth defects, has conducted interviews since 1997 with mothers of infants with congenital malformations and control mothers at several funded Centers across the nation. These ten Centers for Birth Defects Research and Prevention

(CBDRP) include Arkansas, California, Georgia, Iowa, Massachusetts, New York, Texas, New Jersey, North Carolina and Utah.[178]

Study population

The present study focused only on NBDPS control mothers with EDD from 1997 to 2005. The NBDPS controls included mothers who had live births without major birth defects and were residents of one of the geographic areas covered by the CBDRP population registries at the time of delivery. They were randomly sampled from birth certificates (Arkansas, for EDDs after 2000; Georgia, for EDDs after 2000; Iowa; Massachusetts; New Jersey; North Carolina; and Utah) or hospital records (Arkansas, for EDDs prior to 2001; California; Georgia, for EDDs prior to 2001; New York; and Texas).[179] States that selected controls from hospitals utilized a systematic random sampling scheme so that infants selected were in proportion to the number of births at each hospital in the geographic area.[178] Controls were excluded if they had a major birth defect, were not a resident of one of the geographic areas covered by CBDRP, were adopted or in foster care, had a deceased mother, or were stillborn. We included data only on mothers who delivered singleton births since multiple births have been identified as a major risk factor for SGA. Previous studies have shown NBDPS control participants to be generally representative of their base populations.[179] Since the study population includes infants without any major birth defects, it allows a clearer interpretation of the study results because SGA and low birthweight have been observed to be more common in infants with malformations.[180, 181]

Case definition

SGA was defined as infants with birthweight less than 10th percentile for given gestational age, gender, and race/ethnicity. The 10th percentile cut off used for classification of SGA was based on the US singleton birthweight percentiles for gestational age by race, parity, and gender published by Overpeck et al.[182] and Zhang & Bowes.[183] Infants with gestational ages less than 20 weeks or more than 44 weeks were excluded. Information on gestational age and birthweight at delivery were obtained from medical records or birth certificates of the participants. If not available, the following criteria were used for calculation of gestational age: 1) estimated due date reported by mother in the interview; 2) ultrasound <14 weeks; 3) last menstrual period; 4) ultrasound >14 weeks; and/or 5) standard neonatal exam.

Control definition

Controls were defined as infants with birthweight at or greater than 10th percentile for given gestational age, gender, and race/ethnicity. Birthweight percentiles published by Zhang & Bowes and Overpeck et al. were utilized for classification of controls.[182, 183]

Data Collection

The NBDPS utilized a standard procedure for contacting case and control mothers and enrolling them in the study. The mothers were mailed a packet that included an introductory letter describing the study, frequently asked questions,

a ‘Right of Research Subjects’ fact sheet, a $20 money order, a response list, and a calendar that covered the duration of her pregnancy. Approximately 10 days after the packets were mailed, interviewers contacted the mothers to answer any questions, conduct the interview or schedule a more convenient time for the interview. The interview was completed in single or multiple sessions at the mother’s request. Interviews were targeted for completion within 6 months of EDD until 24 months post-delivery. Women were not interviewed until six weeks after the EDD or delivery to reduce recall bias between women with preterm and full term births.

The data collection process was based on the instrument used for the Birth Defects Risk Factor Study, the California Birth Defects Monitoring Program, and the Iowa Birth Defect Registry. The interviews were conducted in English or Spanish by trained female interviewers using a standard questionnaire after oral informed consent was obtained. It took approximately 1-1½ hours to complete and covered topics regarding maternal health (diseases and illnesses, fever, injuries, medications); pregnancy issues (pregnancy history, prenatal care); diet/substance abuse (vitamins, food supplements, dietary assessment, caffeine consumption, tobacco, alcohol, street drugs); home/work (residential addresses, occupation); demographic characteristics; and water use.[178] Most questions were structured with pre-coded response lists and detailed questions about exposure three months prior to conception through the end of pregnancy were asked. The pregnancy calendar allowed mothers to recall exposures by date,

month, or trimester of pregnancy. Data from the NBDPS with EDDs from 1997-2005 had a total of 6807 (66.2%) control mothers who participated in the interview and the median length of time from EDD to interview was 7.7 months.

Assessment of nitrosatable drug exposure (Specific aim 1,3)

As a part of the NBDPS, women were interviewed about prescription and non-prescription drugs taken from three months prior to conception to the end of pregnancy for specific illness and diseases (e.g., asthma, diabetes, hypertension etc.) and about specific products used (e.g., ampicillin, phenytoin, metoprolol). The frequency of use and corresponding dates of usage were also obtained. The Slone Epidemiology Center Drug Dictionary was used to link the reported drugs to their active ingredients.[184] Detailed methods used to classify drugs with respect to nitrosatability, functional groups and indications were described in previous publications.[25, 174] The methodology used for classification included: 1) active ingredients for all orally administered drugs, and orally inhaled medications were identified; 2) these active ingredients were cross referenced against previously compiled lists of nitrosatable medicinal compounds published by Brambilla & Martelli and McKean Cowdin et al.;[89, 185] 3) identified nitrosatable compounds were categorized into nitrosatable groups based on the presence of amine (primary, secondary, tertiary) and amide functional groups in their chemical structure; 4) primary amines were excluded as they do not form stable N-nitroso compounds; 5) a few drugs were categorized into multiple drug groups depending on the functional group present (e.g., atenolol is both a secondary

amine and amide) and finally 6) each nitrosatable drug component was classified by the drug's primary indication (e.g., antihistamine, antiepileptic) and pharmacologic class (e.g., opioid, macrolide).

We assessed nitrosatable drug exposure during the first, second, and third trimester among NBDPS control mothers with EDDs from 1997-2005. The same method was utilized for classification of drugs based on nitrosatability. For each nitrosatable drug component, information on whether the drug was taken and frequency of use was used to estimate exposure during each trimester of pregnancy. The drug components were further categorized into their respective nitrosatable drug groups (secondary amines, tertiary amine, and amides) and drug indication groups.

Dietary assessment of nitrate and nitrite intake (Specific aim 2,3)

A 58-item food frequency questionnaire (FFQ) adapted from the Short Willett Food Frequency Questionnaire was used to collect information on dietary intake of mothers during the year prior to conception. In addition, women were asked about cereal intake and alcohol consumption three months prior to conception through the end of pregnancy. Question regarding intake of avocado, salsa, refried beans, and tortillas food items were also added to the questionnaire to incorporate the diverse diet of the NBDPS population. Studies of validity and reproducibility of the original Willett FFQ indicate that the dietary questionnaire provides useful information about women's nutrient intake over one year period compared to four one week diet records.[186] Furthermore, the

assessment of dietary intake of nutrients using the Willett FFQ was also comparable to nutrient intakes estimated using 24 hour recalls.[187]

The NBDPS nutrient calculations were based on the USDA National Nutrient Database for Standard Reference 19. Daily intake of each food component was calculated based on the serving size or frequency of use. Food items or groups listed in the NBDPS FFQ were assigned codes that correspond with the USDA unique codes. Since the USDA nutrient database did not include estimates of nitrates and nitrites, values were estimated for food items or group in the NBDPS questionnaire by Griesenbeck and colleagues.[188] Dietary nitrates and nitrites estimates were calculated using a multi-step procedure that included 1) food database creation 2) value selection and 3) estimate generation. First, a food database was created to identify nitrate values for each food item or group listed in the NBDPS FFQ based on estimates from published literature either from 1980 to present or 1970-1979. Second, the values of nitrate estimates were ranked depending on the source of published studies either in United States and Canada or other countries with traditionally Western diet. Third, a summary estimate for each food item was calculated as the weighted mean of the published values. Then, for each food item, the nitrate and nitrite estimates were calculated in grams per serving size using the summary estimate and standard serving size. The estimates were then multiplied by the number of servings per month, summed across all food items and then divided by 30 to calculate the average daily nitrate and nitrite intake. Additionally, the estimate for total dietary

nitrite was calculated as [dietary nitrite + (0.05 X nitrate intake)] based on the method suggested by Choi et al.[189] In the present study, we examined the association between dietary nitrates, nitrites, and total nitrites and risk of SGA birth. Tertiles of nitrates, nitrites, and total nitrites were generated based on the control women's distributions and restricted to total caloric intake between 500-5000kcal.

Assessment of vitamin C intake (Specific aim 4)

The NBDPS collects information on vitamin use (single, prenatal, and multivitamins) three months prior to conception through the end of pregnancy. We calculated the intake of vitamin C supplementation during the second and third trimester among NBDPS controls mothers with EDDs from 1997-2005 as was estimated for the first trimester in our previous publication.[174] Vitamin C supplementation was further categorized into none, less than daily, and daily depending on the frequency of intake. Information on dietary intake of vitamin C was obtained from the FFQ and the NBDPS data tools. Estimates for dietary vitamin C were calculated based on the daily estimated dietary intake from the NBDPS Nutrient Database.

Analysis plan and power calculations

<u>Analysis plan</u>

Data for all NBDPS control participants with EDDs from 1997-2005 was used. With respect to specific aim 1, nitrosatable drugs exposure in relation to

SGA births was analyzed in the following ways 1) any drug taken during each trimester or anytime during pregnancy (binary variable yes/no) 2) drugs classified into nitrosatable groups (secondary amines, tertiary amine, amides) using the same exposure periods as in #1 3) nitrosatable drugs groups from #2 categorized by indication or therapeutic class. Women who reported no nitrosatable drug use anytime during pregnancy was used as the referent group. Dietary, nitrates, nitrites, and total nitrites were categorized into tertiles based on the control's women distribution. Unconditional binary logistic regression with adjustments for confounders was used to analyze the association between dietary nitrites and total nitrites with SGA (specific aim 2). All dietary analyses were restricted to participants who reported a daily caloric intake between 500-5000 kcal. These limits were recommended by Willett and have been previously used by dietary studies and those utilizing the NBDPS database.[174, 190-192]

For specific aim 3, we examined whether dietary nitrate or nitrite intake modified the association between nitrosatable drug exposure and SGA. Nitrosatable drug exposure was stratified by tertiles of dietary nitrites/total nitrites. Stratum specific odds ratios and respective 95% confidence intervals were calculated. With respect to specific aim 4, the effect of dietary and supplemental vitamin C on the relation between nitrosatable drug and SGA was analyzed by stratifying by categories of vitamin C supplementation (none, less than daily, daily), and dietary vitamin C intake (<85 mg/day or ≥85 mg/day,

based on NIH recommendation for pregnant women). Odds ratio and 95% confidence limits were obtained for each stratum.

The potential interactions of nitrosatable drugs with dietary nitrites and total nitrites, and vitamin C with nitrosatable drugs as risk for SGA were examined. Multiplicative interaction was assessed by including the product terms of nitrosatable drugs X total dietary nitrite, nitrosatable drugs X vitamin C, and nitrosatable drugs X dietary vitamin C. We tested for additive interaction using a program developed by Andersson et al. that estimated measures of relative excess risk due to interaction (RERI) and attributable proportion due to interaction (AP).[193]

To determine which potential confounding variables to include in the logistic regression models, the following approach was utilized. Covariates were included based on their association with SGA or exposure of interest from previous literature. Maternal race/ethnicity, education, and study center were identified as important predictors of nitrosatable drug use among control participants in the NBDPS in a previous publication and were included in the final model.[25] Forward selection was also utilized for selection of the covariates. The following covariates were considered during the forward selection: maternal race/ethnicity; maternal age at delivery; maternal education; maternal smoking; prepregnancy body mass index; weight gain in kg during pregnancy; parity; infant gender; maternal hypertension before and during pregnancy; and state of residence. Nonsignificant covariates as well as those that did not change the

odds ratio by 10 percent or more were eliminated from the model. Only participants with complete information available for all covariates included in the final logistic models were used for both crude and adjusted analyses.

In the multivariable analyses, the following main effects and interactions were considered:

1. (nitrosatable drug groups) + (dietary nitrites) + (nitrosatable drugs groups) * (dietary nitrites)
2. (nitrosatable drug groups) + (total nitrites) + (nitrosatable drugs groups) * (total nitrites)
3. (nitrosatable drug groups) + (vitamin C supplement) + (nitrosatable drug groups) * (vitamin C supplement)
4. (nitrosatable drug groups) + (dietary vitamin C) + (nitrosatable drug groups) * (dietary vitamin C)

Power calculations

Table 1.1 below shows the minimum detectable odds ratio for risk of SGA births by nitrosatable drug and dietary nitrite exposures. Malik et al. noted the prevalence of SGA infants to be 7.82% among NBDPS controls participants with deliveries from 1997-2002.[194] This was used to estimate the number of SGA births in this study population.

Table 1.1 Minimum Detectable Odds ratio for SGA in Relation to Selected Exposures in NBDPS Controls

Outcomes	Cases	Exposure(s)	Odds ratio	
			Power	
			80%	90%
SGA births	509	Any nitrosatable drugs	1.35	1.41
		Secondary amines	1.45	1.53
		Tertiary amines	1.45	1.53
		Amides	1.56	1.67
		Dietary nitrite or total nitrite	1.31	1.37

Number of controls (births at 10th percentile or above birth weight for gestational age) - 6027
Prevalence of exposure in controls: nitrosatable drugs-23.6%, secondary amines-12.4%, tertiary amines-12.2%, amides-7.6%, dietary nitrite or total nitrite tertiles- 33.33%
Two tailed significance level of 0.05

Potential problems and alternative strategies

The study had several limitations. In NBDPS, participants were interviewed about the frequency of foods consumed a year prior to conception which might have reduced recall accuracy resulting in misclassification of foods consumed during pregnancy. However, the misclassification might have been non-differential with respect to the outcome (SGA) as the same period of dietary assessment was used for all NBDPS participants. Furthermore, studies have indicated that consumption of vegetables and meats, major source of nitrates and nitrites, respectively, did not significantly differ before and during pregnancy, and strong correlations have been reported between vegetable intake at the beginning and end of pregnancy.[13, 195]

Another limitation pertains to potential maternal recall bias of drug

exposures during pregnancy. Since the study utilized exposure data for all NBDPS controls who had births without congenital malformations, the possibility of recall bias is less likely. Previous studies among women with normal or adverse pregnancy outcomes have found little evidence for differential recall of drugs including analgesics, antibiotics, and antinauseants that have nitrosatable drug components.[196, 197] To reduce recall bias, the NBDPS utilized a two-level approach to assess drug use by asking participants about drug use by indication and medication name. This approach has been shown to be more accurate than an open ended questionnaire.[198, 199] Furthermore, women were not aware of nitrosatable drug components in drugs they consumed so recall bias would have been less likely. Information on dose of the drugs was not collected in NBDPS. However, drug dose might not correspond to the quantity of N-nitroso compounds formed since conversion of nitrosatable drugs to N-nitroso compounds is dependent on presence and concentration of nitrite in stomach, gastric pH, reaction time, and other catalysts and inhibitors of the reaction.[89, 200]

Significance

Very few epidemiologic studies have examined the relation of prenatal exposure to nitrosatable drugs and dietary nitrites intake with SGA. The prevalence of nitrosatable drug use during the first trimester of pregnancy was approximately 24% among control participants in the NBDPS from 1997-2005.[25] Only one published study examined the association between nitrosatable drugs use during pregnancy and adverse pregnancy outcomes; a significantly reduced

risk was observed for birthweight (<2000 grams) due to nitrosatable drug exposure.[93] However, the interaction of dietary nitrate/nitrite intake with nitrosatable drugs and possible role of N-nitroso compounds were not assessed in this study.

The mean birthweight of term singleton births in United States decreased by 52 g from 1990-2005.[201] The proportion of neonates born SGA from 1990-2005 remained constant around 10.3%, however in low risk populations the prevalence of SGA slightly increased from 1990 (7.2%) to 2005 (8.1%). Also, infants born SGA are at increased risk for development of several chronic diseases, metabolic disorders and other co-morbidities in adulthood. Despite accumulating evidence on the adverse health effects of being born SGA, the causes and mechanisms for fetal growth restriction are unclear. Findings from this study provide valuable information on the risk of SGA birth associated with maternal dietary consumption of nitrates and nitrites and nitrosatable drug use during pregnancy. If increased risk for SGA is observed, women prescribed medications that have nitrosatable components should be given alternative drugs that are not nitrosatable. Women should be counseled of the potential risk to the baby from use of over the counter medications, some of which may be nitrosatable. They should also be encouraged to take vitamin supplements containing vitamin C that may attenuate the risk. Although exposure to nitrates and nitrites through diet is unavoidable, reduction of nitrosatable drug use during pregnancy might lower the risk of SGA births.

2. NITROSATABLE DRUG EXPOSURE DURING PREGNANCY AND SMALL-FOR-GESTATIONAL-AGE BIRTHS

Overview

Several prescription and non-prescription medications contain nitrosatable amines (secondary or tertiary amines) or amides that are precursors to the formation of N-nitroso compounds. Results from experimental studies on animal models suggest that nitrosamines may reduce birthweight in offspring. Using data from the National Birth Defects Prevention Study (NBDPS) control participants, we examined the relation between prenatal exposure to drugs classified as nitrosatable and small-for-gestational (SGA) births among 526 mothers of infants with birthweight <10th percentile and 5970 mothers of control infants during 1997-2005. Information was collected by telephone interview on type and frequency of medication use, demographic characteristics, and maternal health. Drugs reported were classified according to nitrosatability and primary indication of use. Overall, prenatal use of nitrosatable drugs was not associated with SGA except for a few notable exceptions. Relative to women who reported no nitrosatable drug use anytime during pregnancy, women who took nitrosatable amides during the third trimester of pregnancy were more likely to have SGA births (adjusted odds ratio [OR] 1.43 [95% confidence interval [CI] 1.00, 2.05]). This association was strongest among women exposed to amides during the eighth and ninth month of pregnancy (OR 1.57 [95% CI 1.02, 2.44]

and OR 1.92 [95% CI 1.17, 3.17], respectively). Exposure to tertiary amine drugs including antihistamines during the third trimester and analgesic use during the first trimester was significantly associated with SGA. Findings suggest that maternal exposure to nitrosatable drugs during pregnancy may not be associated with SGA.

Background

Birthweight is considered as an important predictor of perinatal morbidity and mortality. According to the National Vital Statistics report for 2009, infant mortality rates were about 24 times higher (53.05 per 1000) for low birthweight infants (less than 2500 grams) than for infants with birthweight 2500 grams or more.[1] Infants born small-for-gestational-age (SGA) are usually defined as less than 10th percentile of birth weight for gestational age.[2] SGA infants may have a persistent short stature in childhood and adulthood. However, most infants present early postnatal or catch-up growth from birth to two years of age, and rapid weight gain during childhood. They are also at increased risk of developing chronic diseases in adulthood such as cardiovascular disease, insulin resistance, diabetes mellitus, dyslipidemia, and renal disease.[3-6] The causes and mechanisms of SGA are multifactorial. Numerous environmental contaminants including exposure to air pollutants[60, 62, 63, 67-71, 75, 76, 78] and tobacco smoke[47-51] have been associated with SGA; however, no published study has examined the relation of nitrosatable drugs with SGA.

Certain medications classified as containing nitrosatable amines (secondary or tertiary amines) or amides are precursors to the formation of N-nitroso compounds. These compounds can be formed *in vivo* when nitrosatable amines or amides react with nitrosating agents such as nitrite in the acidic environment of the stomach.[24] Endogenous formation of N-nitroso compounds accounts for 45 to 75 percent of the total human exposure; however, exposure may also occur from exogenous sources, such as processed meat products, cured meats, smoked fish, alcohol, and tobacco.[86-88]

N-nitroso compounds, genotoxic compounds including nitrosamines and nitrosamides, are known to cause congenital malformations in animal models,[90, 202-204] and the role of these compounds on fetal weight warrants further research. Some drugs classified as nitrosatable tertiary amines form n-nitrosodimethylamine (NDMA) in the presence of nitrite.[89] Experimental evidence has shown that exposure of pregnant mice to acetoxymethyl-methylnitrosamine, which has the same active intermediate metabolite as NDMA, was associated with a significant increase in number of weight retarded fetuses.[90] Annola et al. detected transplacental transfer of NDMA in perfused human placentas from women who recently delivered full-term babies indicating that maternal exposure to NDMA may possibly affect fetal health.[91] In addition, fetal weight was significantly reduced when pregnant mice were exposed to both ethylenethiourea (a nitrosatable compound) and nitrite but no effect was observed when exposed to these compounds separately.[92] These findings

suggest that N-nitroso compounds might influence fetal growth.

Several prescription and non-prescription drugs that consist of secondary or tertiary amines or amides in their molecular structure are commonly used during pregnancy. Among control participants of the National Birth Defects Prevention Study (NBDPS), approximately 24 percent of the mothers reported use of one or more nitrosatable drugs during the first trimester of pregnancy.[25] In addition, maternal exposure to nitrosatable drugs during one month prior to one month post-conception and first trimester of pregnancy was associated with several birth defects in offspring, including neural tube defects,[174] limb deficiencies, and selected oral clefts and congenital heart defects.[192]

Although numerous epidemiologic studies have examined the role of maternal drug use during pregnancy on birthweight and risk of SGA birth, inconsistent results have been reported. A variety of prescription drugs such as antidepressants,[95-97, 100-102] antidiabetics,[113-115] antiepileptics,[135, 138, 139, 141] beta lactam antibiotics,[163-165] specific cardiovascular medications,[119, 121, 123, 145, 146] and drugs used for the treatment of asthma,[106-109] migraine,[131-133] and infections[166-168] were found to be associated with SGA. A few over the counter medications including antiemetics,[152, 154] decongestants,[110] nicotine replacements,[156, 157] and drugs indicated for acid reflux disease[127, 128] were also linked with SGA. But none of the studies evaluated the risk of SGA in relation to drugs classified as nitrosatable.

Only one published study has investigated the association between

nitrosatable drugs and birthweight. Using data from the Collaborative Perinatal Project, Olshan and Faustman examined adverse pregnancy outcomes among 6,061 mothers exposed to nitrosatable drugs anytime during pregnancy, with 1,775 being exposed during the first four months, and 6,921 mothers were not exposed to these drugs. Exposure to nitrosatable drugs during the given time periods was associated with a significantly lower risk for low birthweight (<2000 grams).[93]

Based on the previous animal and human evidence of the effects of nitrosatable compounds or drugs on fetal birthweight, we examined the relation between SGA and maternal exposure to nitrosatable drugs anytime during pregnancy or by each trimester of pregnancy. The nitrosatable drugs were classified by the type of functional group (secondary amines, tertiary amines, or amides) and indication of use.

Methods

To investigate the association between prenatal exposure to nitrosatable drugs and SGA birth, we used data from control participants of the National Birth Defects Prevention Study (NBDPS). The NBDPS is a large population-based case control study of birth defects in the United States. Since the study's inception in 1997, ten Centers for Birth Defects Research and Prevention (CBDRP) including Arkansas, California, Georgia, Iowa, Massachusetts, New York, and Texas (from 1998 to present); New Jersey (from 1998 to 2002); and North Carolina and Utah (from 2003 to present) have participated in the study.[178]

Study population

We focused on NBDPS control mothers who had live births without major birth defects with estimated dates of delivery (EDDs) between October 1, 1997, and December 31, 2005; and were residents of one of the geographic areas covered by the CBDRP population registries. They were randomly sampled from either birth certificates (Arkansas, for EDDs after 2000; Georgia, for EDDs after 2000; Iowa; Massachusetts; New Jersey; North Carolina; and Utah) or hospital records (Arkansas, for EDDs prior to 2001; California; Georgia, for EDDs prior to 2001; New York; and Texas).[179] States that selected controls from hospitals utilized a systematic random sampling scheme so that infants selected were in proportion to the number of births at each hospital in the geographic area.[178] The controls-infants were ineligible if they were stillborn, had a major birth defect, were adopted or in foster care, had a deceased mother, or were born outside the study area. We included data only on mothers who delivered singleton births since multiple births have been identified as a major risk factor for SGA. The institutional review boards in each state and the Center for Disease Control and Prevention approved the NBDPS protocol, and the institutional review board of Texas A&M University approved this study.

Case and control definition

Cases were defined as infants with birthweight less than the 10^{th} percentile for given gestational age, gender, and race/ethnicity. Infants with birthweight at or greater than 10^{th} percentile were identified as controls. The US

singleton birthweight percentiles for gestational age by maternal race, parity, and infant gender, published by Overpeck et al.[182] and Zhang & Bowes,[183] were used for classification of SGA. Infant with gestational ages less than 20 weeks or more than 44 weeks were excluded. In NBDPS, information on gestational age and birthweight at delivery were obtained from medical records or birth certificates of the participants. If not available, the following criteria was used for calculation of gestational age: 1) estimated due date reported by mother in the interview; 2) ultrasound <14 weeks; 3) last menstrual period; 4) ultrasound >14 weeks; or 5) standard neonatal exam.

Data collection

The NBDPS utilized a standard procedure for contacting the mothers and enrolling them in the study. The interviews were targeted for completion within six months of EDD until 24 months post-delivery. In the original study, women were not interviewed until six weeks after the EDD or actual date of delivery to reduce recall bias between women with preterm and full term births. After oral informed consent was obtained, the interviews were conducted either in English or Spanish by trained female interviewers using a computer-assisted telephone interview. It took approximately 1-1½ hours to complete and covered topics regarding maternal health (including medications taken); pregnancy issues; diet/substance abuse; work history; demographic characteristics; and water use.[178] Most questions were structured with pre-coded response lists and detailed questions about drug exposure from three months prior to conception

through the end of pregnancy were asked. A pregnancy calendar was provided that helped mothers to recollect exposures by date, month, or trimester of pregnancy. Data from the NBDPS with EDDs from 1997-2005 had a total of 6807 (66.2%) control mothers who participated in the interview and the median length of time from EDD to interview was 7.7 months.

Classification of nitrosatable drugs

As a part of the NBDPS interview, women were questioned about prescription and non-prescription drugs taken (medication name), the corresponding dates and frequency of use from three months prior to conception to the date of birth of index pregnancy. Information on drugs used for specific illness and diseases (e.g., asthma, diabetes, hypertension etc.), and about specific products (e.g., ampicillin, phenytoin, metoprolol) were collected. The Slone Epidemiology Center Drug Dictionary was used to link the reported drugs to their active ingredients.[184]

Detailed methods used to classify drugs with respect to nitrosatability, functional groups, and indications were described in previous publications.[25, 174] The methodology used for classification included: 1) active ingredients for all orally administered drugs, and orally inhaled medications were identified; 2) these active ingredients were cross referenced with a comprehensive list of nitrosatable medicinal compounds published by Brambilla & Martelli[89] and McKean Cowdin et al.;[185] 3) identified nitrosatable compounds were categorized based on the presence of amine (secondary or tertiary) and amide functional

groups; and further 5) each nitrosatable drug component was classified by its primary indication (e.g., antihistamine, antiepileptic) and pharmacologic class (e.g., opioid, macrolide). A few drugs were categorized into multiple nitrosatable groups (e.g., atenolol is both a secondary amine and amide).

In this study, we focused on maternal exposure to nitrosatable drugs anytime or by each trimester (first, second, or third) of pregnancy. For each nitrosatable drug component, information on whether the drug was taken and frequency of use was used to estimate exposure for each month and trimester of pregnancy. Complete information on nitrosatable drug use anytime during pregnancy and covariates was available for 99.0% and 98.5% of participating case and control women.

Statistical analyses

Data for all NBDPS control participants with EDDs from 1997-2005 were analyzed. The distributions of maternal characteristics were compared between case and control mothers using chi-squared test and univariate analyses were performed to examine the relation between each maternal risk factor and SGA. Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals for SGA in relation to any nitrosatable drug use anytime during pregnancy or by each trimester or month of pregnancy. The specific nitrosatable drugs components (secondary amines, tertiary amines, or amides), and nitrosatable drugs categorized by indication of use or therapeutic class were analyzed separately for their association with SGA using the same exposure

periods. Women who reported no nitrosatable drug use anytime during pregnancy was used as the referent group. All analyses were performed using STATA 11.[205]

Covariates included in the logistic models were selected based on their association with SGA and maternal risk factors associated with nitrosatable drugs use from previous literature. Maternal race/ethnicity, education, and study center were important predictors of nitrosatable drug use among control participants of NBDPS as noted in a previous publication.[25] Non-significant covariates as well as those that did not change the odds ratio by 10 percent or more were eliminated from the final model using forward selection. The following covariates were included in the final model: maternal race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, Asian/Pacific Islander, other), maternal education (<12 years, 12 years, 13-15 years, >15 years), study center (Arkansas, Atlanta, California, Iowa, Massachusetts, New Jersey, New York, North Carolina, Texas, and Utah), maternal smoking (yes, no), and chronic hypertension prior to pregnancy (yes, no). Only participants with complete information available for all covariates included in the final logistic models were used for both crude and adjusted analyses.

A sensitivity analysis was performed for full term case and control infants with gestational ages restricted between 37 and 41 weeks. The above logistic regression analyses were repeated. We also examined the association between nitrosatable drugs and SGA among a subgroup of participants with cases

defined at less than 10th percentile and controls classified using birthweight cut offs at 50th percentile or more, published by Overpeck et al.[182] and Zhang & Bowes.[183]

Results

A total of 526 infants classified as SGA and 5,970 controls-infants with an EDD from 1997-2005 participated in the NBDPS. The median length of time from EDD to interview was eight months for both case and control participants. Compared to control mothers, case mothers were more likely to be Hispanic or Asian/Pacific Islander, had less than 12 years of education, delivered between 20-24 years of age, and reported smoking during pregnancy (Table 2.1). A higher percentage of case mothers (10.5%) had body mass index (BMI) less than 18.5 kg/m^3 than control mothers (4.8%). Case mothers were also more likely to have a lower gestational weight gain of <25lbs (34.0%) compared to control mothers (25.0%). The distributions of maternal characteristics including race/ethnicity, education, age at delivery, study center, BMI, smoking, gender, and gestational weight gain were significantly different between case and control mothers.

Prenatal use of nitrosatable drugs anytime during pregnancy was not found to be associated with SGA (OR 0.92 [95% CI 0.76, 1.12]) (Table 2.2). However, stronger associations were observed with SGA among women who reported nitrosatable drug usage during the third than the first or second trimester of pregnancy. Specifically women who took drugs classified as nitrosatable

Table 2.1 Selected Maternal Characteristics of Small-For-Gestational-Age Infants (Cases) and Controls in the National Birth Defects Prevention Study, 1997-2005

Characteristics of Participants	Controls n=5,970		Cases n=526		OR	95% CI
	No.	%	No.	%		
Race-ethnicity*						
Non-Hispanic white	3573	59.9	289	54.9	1.00	Referent
Non-Hispanic black	685	11.5	44	8.4	0.79	0.57, 1.10
Hispanic	1310	21.9	141	26.8	**1.33**	**1.08, 1.64**
Asian/Pacific Islander	165	2.8	27	5.1	**2.02**	**1.32, 3.09**
All others	237	4.0	25	4.8	1.30	0.85, 2.00
Missing	0	0	0	0	-	-
Education (years)*						
>15	1882	31.5	114	21.7	1.00	Referent
13-15	1597	26.8	142	27.0	**1.47**	**1.14, 1.89**
12	1448	24.3	140	26.6	**1.60**	**1.23, 2.06**
<12	960	16.1	126	24.0	**2.17**	**1.66, 2.82**
Missing	83	1.4	4	0.8	-	-
Age at delivery (years)*						
<18	219	3.7	22	4.2	0.91	0.57, 1.46
18-19	420	7.0	42	8.0	0.91	0.64, 1.30
20-24	1356	22.7	149	28.3	1.00	Referent
25-29	1601	26.8	133	25.3	0.76	0.59, 0.97
30-34	1569	26.3	98	18.6	0.57	0.44, 0.74
>34	805	13.5	82	15.6	0.93	0.70, 1.23
Study center*						
Arkansas	747	12.5	78	14.8	1.00	Referent
California	760	12.7	62	11.8	0.78	0.55, 1.11
Georgia	674	11.3	55	10.5	0.78	0.54, 1.12
Iowa	742	12.4	62	11.8	0.80	0.56, 1.13
Massachusetts	492	8.2	49	9.3	0.95	0.66, 1.39
North Carolina	539	9.0	46	8.8	0.82	0.56, 1.20
New Jersey	675	11.3	82	15.6	1.16	0.84, 1.61
New York	648	10.9	44	8.4	0.65	0.44, 0.95
Texas	360	6.0	23	4.4	0.61	0.38, 0.99
Utah	333	5.6	25	4.8	0.72	0.45, 1.15
Body mass index (kg/m^2)*						
<18.5	289	4.8	55	10.5	**2.08**	**1.52, 2.84**
18.5–24.9	3205	53.7	293	55.7	1.00	Referent
25.0–29.9	1308	21.9	95	18.1	0.79	0.62, 1.01
>29.9	948	15.9	54	10.3	0.62	0.46, 0.84
Missing	220	3.7	29	5.5	-	-
Smoking*						
No	4809	81.5	392	75.0	1.00	Referent
Yes	1094	18.5	131	25.0	**1.47**	**1.19, 1.81**
Missing	0	0	0	0	-	-
Gender*						
Male	2992	50.1	297	56.5	1.00	Referent
Female	2978	49.9	229	43.5	**1.29**	**1.08, 1.54**
Missing	0	0	0	0	-	-

Table 2.1 Continued.

Characteristics of Participants	Controls n=5,970		Cases n=526		OR	95% CI
	No.	%	No.	%		
Parity						
Nulliparous	2392	40.1	204	38.8	1.00	Referent
Primiparous	1993	33.4	187	35.6	1.10	0.89, 1.35
Multiparous	1585	26.6	135	25.7	1.00	0.80, 1.25
Gestational weight gain*						
<25lbs	1492	25.0	179	34.0	**1.27**	**1.03, 1.57**
25-35lbs	2088	35.0	197	37.5	1.00	Referent
>35lbs	2154	36.1	127	24.1	0.62	0.50, 0.79
Missing	236	4.0	23	4.4	-	-

Abbreviations: OR, odds ratio; CI, confidence interval.

* $p < 0.05$; statistically significant difference in distribution between cases and controls participants

Table 2.2 Exposure to Nitrosatable Drugs During Pregnancy and Risk of Small-For-Gestational-Age Births, National Birth Defects Prevention Study, 1997-2005

Timing of drug exposure	Type of drug exposure	Cases		Controls		Unadjusted		Adjusted	
		No.	%[a]	No.	%[a]	OR[b]	95% CI	OR[b,c]	95% CI
Anytime during pregnancy	No nitrosatable drugs	338	64.9	3620	61.6	1.00	Referent	1.00	Referent
	Any nitrosatable	183	35.1	2258	38.4	0.87	0.72, 1.05	0.92	0.76, 1.12
	Secondary amines	93	21.6	1295	26.4	0.77	0.61, 0.98	0.85	0.67, 1.09
	Tertiary amines	86	20.3	1138	23.9	0.81	0.63, 1.04	0.86	0.66, 1.11
	Amides	77	18.6	877	19.5	0.94	0.73, 1.22	0.96	0.74, 1.25
First trimester	No nitrosatable drugs	338	75.6	3620	72.4	1.00	Referent	1.00	Referent
	Any nitrosatable	109	24.4	1382	27.6	0.84	0.67, 1.06	0.91	0.72, 1.15
	Secondary amines	54	13.8	735	16.9	0.79	0.58, 1.06	0.86	0.63, 1.17
	Tertiary amines	47	12.2	724	16.7	0.70	0.51, 0.95	0.76	0.55, 1.05
	Amides	36	9.6	449	11.0	0.86	0.60, 1.23	0.90	0.63, 1.30
Second trimester	No nitrosatable drugs	338	75.1	3620	73.8	1.00	Referent	1.00	Referent
	Any nitrosatable	112	24.9	1283	26.2	0.93	0.75, 1.17	1.00	0.79, 1.26
	Secondary amines	59	14.9	755	17.3	0.84	0.63, 1.12	0.93	0.69, 1.25
	Tertiary amines	52	13.3	596	14.1	0.93	0.69, 1.27	0.98	0.72, 1.35
	Amides	38	10.1	392	9.8	1.04	0.73, 1.48	1.07	0.75, 1.53
Third trimester	No nitrosatable drugs	338	77.0	3620	76.0	1.00	Referent	1.00	Referent
	Any nitrosatable	101	23.0	1143	24.0	0.95	0.75, 1.19	1.02	0.80, 1.29
	Secondary amines	50	12.9	683	15.9	0.78	0.58, 1.07	0.87	0.63, 1.19
	Tertiary amines	45	11.8	490	11.9	0.98	0.71, 1.36	1.04	0.74, 1.46
	Amides	40	10.6	328	8.3	1.31	0.92, 1.85	1.43	1.00, 2.05

Abbreviations: OR, odds ratio; CI, confidence interval.

Table 2.2 Continued.

[a]Percentages for no nitrosatable drug exposure are based on total participants with complete information whereas percentages for secondary or tertiary amines or amides includes complete information for the given drug group and excludes other nitrosatable groups in the denominator.

[b]Crude and adjusted odds ratios include only cases and controls with complete information for drug exposures and covariates.

[c]Adjusted for maternal race/ethnicity, education, smoking, chronic hypertension, and study center.

Table 2.3 Exposure to Nitrosatable Drugs During Pregnancy and Risk of Full Term[a] Small-For-Gestational-Age Births, National Birth Defects Prevention Study, 1997-2005

Timing of drug exposure	Type of drug exposure	Cases		Controls		Unadjusted		Adjusted	
		No.	%[b]	No.	%[b]	OR[c]	95% CI	OR[c,d]	95% CI
Anytime during pregnancy	No nitrosatable drugs	306	63.8	3290	61.9	1.00	Referent	1.00	Referent
	Any nitrosatable	174	36.2	2027	38.1	0.92	0.76, 1.12	0.97	0.79, 1.19
	Secondary amines	86	21.9	1154	26.0	0.80	0.62, 1.03	0.88	0.68, 1.14
	Tertiary amines	81	20.9	1023	23.7	0.85	0.66, 1.10	0.90	0.69, 1.18
	Amides	74	19.5	784	19.2	1.01	0.78, 1.32	1.04	0.79, 1.36
First trimester	No nitrosatable drugs	306	74.5	3290	72.6	1.00	Referent	1.00	Referent
	Any nitrosatable	105	25.5	1241	27.4	0.91	0.72, 1.15	0.98	0.77, 1.25
	Secondary amines	52	14.5	654	16.6	0.85	0.63, 1.16	0.94	0.68, 1.29
	Tertiary amines	46	13.1	656	16.6	0.75	0.55, 1.04	0.82	0.59, 1.14
	Amides	34	10.0	400	10.8	0.91	0.63, 1.32	0.97	0.66, 1.42
Second trimester	No nitrosatable drugs	306	74.3	3290	74.3	1.00	Referent	1.00	Referent
	Any nitrosatable	106	25.7	1140	25.7	1.00	0.79, 1.26	1.06	0.83, 1.35
	Secondary amines	53	14.8	672	17.0	0.85	0.63, 1.15	0.94	0.69, 1.29
	Tertiary amines	48	13.6	529	13.9	0.98	0.71, 1.34	1.03	0.74, 1.44
	Amides	38	11.1	348	9.6	1.17	0.82, 1.67	1.21	0.84, 1.75
Third trimester	No nitrosatable drugs	306	75.6	3290	76.4	1.00	Referent	1.00	Referent
	Any nitrosatable	99	24.4	1016	23.6	1.05	0.83, 1.33	1.13	0.88, 1.44
	Secondary amines	48	13.6	605	15.5	0.85	0.62, 1.17	0.95	0.68, 1.31
	Tertiary amines	43	12.3	439	11.8	1.05	0.75, 1.47	1.12	0.79, 1.58
	Amides	39	11.3	289	8.1	1.45	1.02, 2.07	1.61	1.11, 2.33

Abbreviations: OR, odds ratio; CI, confidence interval

Table 2.3 Continued.

[a]Data was restricted to participants with gestational ages between 37 and 41 weeks.

[b]Percentages for no nitrosatable drug exposure are based on total participants with complete information whereas percentages for secondary or tertiary amines or amides includes complete information for the given drug group and excludes other nitrosatable groups in the denominator.

[c]Crude and adjusted odds ratios include only cases and controls with complete information for drug exposures and covariates.

[d]Adjusted for maternal race/ethnicity, education, smoking, chronic hypertension, and study center.

amides during the third trimester of pregnancy were more likely to have SGA infants compared to women who did not take nitrosatable drugs anytime during pregnancy (OR 1.43 [95% CI 1.00, 2.05]). When analyses were restricted to full term case and control-births, a stronger association was observed between amide drug exposure during the third trimester of pregnancy and SGA (OR 1.61 [95% CI 1.11, 2.33]) (Table 2.3). Focusing on nitrosatable drug exposure by each month of pregnancy, we found that women who reported nitrosatable amide use during the eighth and ninth month of pregnancy were more likely to have SGA births (OR 1.57 [95% CI 1.02, 2.44]; and OR 1.92 [95% CI 1.17, 3.17], respectively) (Table 2.4). Similar findings were observed for full term case and control-infants (OR 1.75 [95% CI 1.11, 2.74]; and OR 1.91 [95% CI 1.15, 3.20], respectively) with prenatal use of amides during the eighth and ninth month of pregnancy (Table 2.5). Exposure to nitrosatable amides during the sixth month of pregnancy was also significantly associated with SGA (OR 1.74 [95% CI 1.10, 2.76]) (Table 2.5).

As part of a sensitivity analyses, we examined the relation between maternal exposure to nitrosatable drugs birthweight cut offs at 50th percentile or more. Although, the associations observed did not change the overall conclusion, stronger associations were noted between exposure to amides during the third trimester of pregnancy and SGA (OR 1.53 [95% CI 1.04, 2.25]). Higher ORs were also observed for SGA with amide drug exposure during the eighth and ninth month of pregnancy (ORs 1.62 [95% CI 1.02, 2.59]); and OR

Table 2.4 Exposure to Nitrosatable Drugs by Each Month of Pregnancy and Risk of Small-For-Gestational-Age Births, National Birth Defects Prevention Study, 1997-2005

Timing of drug exposure	Type of drug exposure	Cases		Controls		Unadjusted		Adjusted	
		No.	%[a]	No.	%[a]	OR[b]	95% CI	OR[b,c]	95% CI
1st month	No nitrosatable drugs	338	83.0	3620	81.8	1.00	Referent	1.00	Referent
	Any nitrosatable	69	17.0	807	18.2	0.92	0.70, 1.20	0.98	0.74, 1.29
	Secondary amines	39	10.3	488	11.9	0.86	0.61, 1.21	0.92	0.65, 1.31
	Tertiary amines	35	9.4	385	9.6	0.97	0.68, 1.40	1.06	0.73, 1.54
	Amides	18	5.1	217	5.7	0.89	0.54, 1.46	0.93	0.56, 1.54
2nd month	No nitrosatable drugs	338	82.4	3620	80.7	1.00	Referent	1.00	Referent
	Any nitrosatable	72	17.6	865	19.3	0.89	0.68, 1.16	0.96	0.73, 1.26
	Secondary amines	31	8.4	474	11.6	0.70	0.48, 1.02	0.76	0.51, 1.12
	Tertiary amines	33	8.9	430	10.6	0.82	0.57, 1.19	0.88	0.60, 1.29
	Amides	26	7.1	220	5.7	1.27	0.83, 1.93	1.33	0.87, 2.05
3rd month	No nitrosatable drugs	338	82.6	3620	80.4	1.00	Referent	1.00	Referent
	Any nitrosatable	71	17.4	880	19.6	0.86	0.66, 1.13	0.95	0.72, 1.26
	Secondary amines	41	10.8	460	11.3	0.95	0.68, 1.34	1.05	0.74, 1.48
	Tertiary amines	31	8.4	450	11.1	0.74	0.50, 1.08	0.82	0.55, 1.21
	Amides	17	4.8	228	5.9	0.80	0.48, 1.32	0.92	0.55, 1.54
4th month	No nitrosatable drugs	338	82.6	3620	80.9	1.00	Referent	1.00	Referent
	Any nitrosatable	71	17.4	856	19.1	0.89	0.68, 1.16	0.98	0.74, 1.29
	Secondary amines	40	10.6	512	12.4	0.84	0.60, 1.18	0.93	0.66, 1.33
	Tertiary amines	31	8.4	411	10.2	0.81	0.55, 1.18	0.89	0.60, 1.32
	Amides	20	5.6	218	5.7	0.98	0.61, 1.57	1.04	0.65, 1.68

Table 2.4 Continued.

Timing of drug exposure	Type of drug exposure	Cases		Controls		Unadjusted OR[b]	95% CI	Adjusted OR[b,c]	95% CI
		No	%[a]	No	%[a]				
5th month	No nitrosatable drugs	338	80.7	3620	81.3	1.00	Referent	1.00	Referent
	Any nitrosatable	81	19.3	833	18.7	1.04	0.81, 1.34	1.11	0.86, 1.45
	Secondary amines	43	11.3	509	12.3	0.90	0.65, 1.26	0.99	0.71, 1.40
	Tertiary amines	37	9.9	371	9.3	1.07	0.75, 1.52	1.13	0.78, 1.63
	Amides	24	6.6	208	5.4	1.24	0.80, 1.91	1.32	0.84, 2.06
6th month	No nitrosatable drugs	338	80.7	3620	81.2	1.00	Referent	1.00	Referent
	Any nitrosatable	81	19.3	837	18.8	1.04	0.80, 1.34	1.10	0.84, 1.42
	Secondary amines	45	11.8	514	12.4	0.94	0.68, 1.30	1.02	0.73, 1.42
	Tertiary amines	38	10.1	380	9.5	1.07	0.75, 1.52	1.09	0.76, 1.57
	Amides	24	6.6	191	5.0	1.35	0.87, 2.09	1.48	0.94, 2.32
7th month	No nitrosatable drugs	338	82.4	3620	80.3	1.00	Referent	1.00	Referent
	Any nitrosatable	72	17.6	890	19.7	0.87	0.66, 1.13	0.94	0.71, 1.23
	Secondary amines	41	10.8	547	13.1	0.80	0.57, 1.12	0.89	0.63, 1.26
	Tertiary amines	33	8.9	380	9.5	0.93	0.64, 1.35	0.97	0.66, 1.43
	Amides	23	6.4	217	5.7	1.14	0.73, 1.77	1.18	0.75, 1.85
8th month	No nitrosatable drugs	338	82.2	3620	81.5	1.00	Referent	1.00	Referent
	Any nitrosatable	73	17.8	822	18.5	0.95	0.73, 1.24	1.03	0.79, 1.36
	Secondary amines	40	10.6	527	12.7	0.81	0.58, 1.14	0.90	0.64, 1.28
	Tertiary amines	32	8.7	367	9.2	0.93	0.64, 1.36	1.00	0.68, 1.48
	Amides	26	7.1	181	4.8	1.54	1.00, 2.36	**1.57**	**1.02, 2.44**
9th month	No nitrosatable drugs	338	84.1	3620	85.0	1.00	Referent	1.00	Referent
	Any nitrosatable	64	15.9	640	15.0	1.07	0.81, 1.42	1.17	0.87, 1.56

Table 2.4 Continued.

Timing of drug exposure	Type of drug exposure	Cases		Controls		Unadjusted		Adjusted	
		No	%[a]	No	%[a]	OR[b]	95% CI	OR[b,c]	95% CI
	Secondary amines	36	9.6	405	10.1	0.95	0.67, 1.36	1.06	0.73, 1.53
	Tertiary amines	30	8.2	286	7.3	1.12	0.76, 1.66	1.23	0.82, 1.84
	Amides	20	5.6	130	3.5	**1.65**	**1.02, 2.67**	**1.92**	**1.17, 3.17**

Abbreviations: OR, odds ratio; CI, confidence interval.

[a]Percentages for no nitrosatable drug exposure are based on total participants with complete information whereas percentages for secondary or tertiary amines or amides includes complete information for the given drug group and excludes other nitrosatable groups in the denominator.

[b]Crude and adjusted odds ratios include only cases and controls with complete information for drug exposures and covariates.

[c]Adjusted for maternal race/ethnicity, education, smoking, chronic hypertension, and study center.

Table 2.5 Exposure to Nitrosatable Drugs by Each Month of Pregnancy and Risk of Full Term[a] Small-For-Gestational-Age Births, National Birth Defects Prevention Study, 1997-2005

Timing of exposure	Type of drug exposure	Cases		Controls		Unadjusted		Adjusted	
		No.	%[b]	No.	%[b]	OR[c]	95% CI	OR[c,d]	95% CI
1st month	No nitrosatable drugs	306	81.8	3290	82.1	1.00	Referent	1.00	Referent
	Any nitrosatable	68	18.2	718	17.9	1.02	0.77, 1.34	1.10	0.82, 1.46
	Secondary amines	38	11.1	436	11.7	0.94	0.66, 1.33	1.01	0.71, 1.45
	Tertiary amines	34	10.0	349	9.6	1.05	0.72, 1.52	1.15	0.78, 1.68
	Amides	18	5.6	188	5.4	1.03	0.63, 1.69	1.10	0.66, 1.83
2nd month	No nitrosatable drugs	306	81.6	3290	80.8	1.00	Referent	1.00	Referent
	Any nitrosatable	69	18.4	780	19.2	0.95	0.72, 1.25	1.02	0.77, 1.35
	Secondary amines	30	8.9	418	11.3	0.77	0.52, 1.14	0.84	0.56, 1.25
	Tertiary amines	32	9.5	393	10.7	0.88	0.60, 1.28	0.93	0.63, 1.38
	Amides	24	7.3	205	5.9	1.26	0.81, 1.95	1.33	0.85, 2.08
3rd month	No nitrosatable drugs	306	81.6	3290	80.6	1.00	Referent	1.00	Referent
	Any nitrosatable	69	18.4	790	19.4	0.94	0.72, 1.23	1.04	0.78, 1.38
	Secondary amines	39	11.3	408	11.0	1.03	0.73, 1.46	1.13	0.79, 1.62
	Tertiary amines	30	8.9	407	11.0	0.79	0.54, 1.17	0.88	0.59, 1.31
	Amides	17	5.3	207	5.9	0.88	0.53, 1.47	1.03	0.61, 1.74
4th month	No nitrosatable drugs	306	82.0	3290	81.1	1.00	Referent	1.00	Referent
	Any nitrosatable	67	18.0	765	18.9	0.94	0.71, 1.24	1.04	0.78, 1.38
	Secondary amines	36	10.5	455	12.2	0.85	0.59, 1.22	0.95	0.66, 1.38
	Tertiary amines	27	8.1	371	10.1	0.78	0.52, 1.18	0.86	0.57, 1.31
	Amides	20	6.1	195	5.6	1.10	0.69, 1.77	1.20	0.74, 1.94

Table 2.5 Continued.

Timing of exposure	Type of drug exposure	Cases		Controls		Unadjusted		Adjusted	
		No	%[b]	No	%[b]	OR[c]	95% CI	OR[c,d]	95% CI
5th month	No nitrosatable drugs	306	79.9	3290	81.6	1.00	Referent	1.00	Referent
	Any nitrosatable	77	20.1	740	18.4	1.12	0.86, 1.45	1.19	0.91, 1.57
	Secondary amines	39	11.3	450	12.0	0.93	0.66, 1.32	1.03	0.72, 1.47
	Tertiary amines	34	10.0	329	9.1	1.11	0.77, 1.61	1.18	0.80, 1.73
	Amides	24	7.3	186	5.4	1.39	0.89, 2.16	1.49	0.95, 2.35
6th month	No nitrosatable drugs	306	79.9	3290	81.8	1.00	Referent	1.00	Referent
	Any nitrosatable	77	20.1	733	18.2	1.13	0.87, 1.47	1.19	0.90, 1.56
	Secondary amines	41	11.8	455	12.2	0.97	0.69, 1.36	1.05	0.74, 1.49
	Tertiary amines	35	10.3	333	9.2	1.13	0.78, 1.63	1.16	0.79, 1.70
	Amides	24	7.3	164	4.8	**1.57**	**1.01, 2.45**	**1.74**	**1.10, 2.76**
7th month	No nitrosatable drugs	306	81.4	3290	80.8	1.00	Referent	1.00	Referent
	Any nitrosatable	70	18.2	783	19.2	0.96	0.73, 1.26	1.04	0.79, 1.38
	Secondary amines	39	11.3	484	12.8	0.87	0.61, 1.23	0.96	0.67, 1.38
	Tertiary amines	31	9.2	336	9.3	0.99	0.67, 1.46	1.05	0.70, 1.56
	Amides	22	6.7	192	5.5	1.23	078, 1.94	1.30	0.82, 2.07
8th month	No nitrosatable drugs	306	81.2	3290	81.7	1.00	Referent	1.00	Referent
	Any nitrosatable	71	18.8	735	18.3	1.04	0.79, 1.36	1.13	0.86, 1.50
	Secondary amines	38	11.1	465	12.4	0.88	0.62, 1.25	0.98	0.68, 1.40
	Tertiary amines	30	8.9	333	9.2	0.97	0.65, 1.43	1.05	0.70, 1.57
	Amides	25	7.6	158	4.6	1.70	1.10, 2.64	**1.75**	**1.11, 2.74**

Table 2.5 Continued.

Timing of exposure	Type of drug exposure	Cases		Controls		Unadjusted		Adjusted	
		No	%[b]	No	%[b]	OR[c]	95% CI	OR[c,d]	95% CI
9th month	No nitrosatable drugs	306	82.9	3290	84.3	1.00	Referent	1.00	Referent
	Any nitrosatable	63	17.1	614	15.7	1.10	0.83, 1.47	1.20	0.89, 1.61
	Secondary amines	35	10.3	388	10.6	0.97	0.67, 1.40	1.08	0.74, 1.56
	Tertiary amines	29	8.7	277	7.8	1.13	0.75, 1.68	1.24	0.82, 1.87
	Amides	19	5.9	124	3.6	1.65	1.00, 2.71	**1.91**	**1.15, 3.20**

Abbreviations: OR, odds ratio; CI, confidence interval.

[a]Data was restricted to participants with gestational ages between 37 and 41 weeks.

[b]Percentages for no nitrosatable drug exposure are based on total participants with complete information whereas percentages for secondary or tertiary amines or amides includes complete information for the given drug group and excludes other nitrosatable groups in the denominator.

[c]Crude and adjusted odds ratios include only cases and controls with complete information for drug exposures and covariates.

[d]Adjusted for maternal race/ethnicity, education, smoking, chronic hypertension, and study center.

2.05 [95% CI 1.19, 3.52], respectively) (data not shown).

With classification of nitrosatable drugs by indication of use, SGA was associated with tertiary amines across several types of indications such as analgesics, antiemetics, antihistamines and cough medications (Table 2.6). Case women were more likely than control women to have reported taken nitrosatable antihistamines during the third trimester of pregnancy (OR 1.63 [95% CI 1.03, 2.56]). Mothers who reported use of analgesics, especially analgesic opioids, during the first trimester were more likely to have full term SGA births compared to mothers who did not report taking these drugs anytime during pregnancy (OR 2.40 [95% CI 1.26, 4.59]; and OR 2.36 [95% CI 1.20, 4.64], respectively). Certain secondary amine drugs indicated for asthma and depression were associated with SGA. Use of asthma tocolytic agents during the second trimester was significantly associated with full term SGA births (OR 3.25 [95% CI 1.28, 8.23]). Additionally, SGA was also associated with anti-infectives and cardiovascular medications classified as nitrosatable amides. Although the confidence intervals were wide, a higher unadjusted OR was noted for SGA with exposure to drugs indicated for cardiovascular disorders, during the second or third trimester of pregnancy (OR 4.03 [95% CI 1.06, 15.28]).

Table 2.6 Exposure to Nitrosatable Drugs Classified by Indication of Use and/or Pharmacologic Class and Risk of Full Term[a] Small-For-Gestational Age Births, National Birth Defects Prevention Study, 1997-2005

Type of drug exposure	Drug indication/ pharmacologic class[b]	Timing of drug exposure	Cases		Controls		Unadjusted OR[c]	95% CI	Adjusted OR[c,d]	95% CI
			No.	%	No.	%				
Secondary amines	Asthma	P1P3	13	4.1	138	4.0	1.01	0.57, 1.81	1.05	0.58, 1.88
		P4P6	22	6.7	164	4.8	1.44	0.91, 2.29	1.51	0.95, 2.42
		P7P9	19	5.9	198	5.7	1.03	0.64, 1.68	1.12	0.68, 1.83
	Asthma beta-adrenergic	P1P3	12	3.8	136	4.0	0.95	0.52, 1.73	0.98	0.53, 1.80
		P4P6	16	5.0	143	4.2	1.20	0.71, 2.04	1.26	0.73, 2.15
		P7P9	12	3.8	144	4.2	0.90	0.49, 1.63	0.95	0.51, 1.74
	Asthma tocolytic	P1P3	1	0.3	2	0.1	5.38	0.49, 59.5	5.52	0.49, 62.2
		P4P6	6	1.9	22	0.7	**2.93**	**1.18, 7.29**	**3.25**	**1.28, 8.23**
		P7P9	8	2.5	55	1.6	1.56	0.74, 3.31	1.79	0.83, 3.83
	Decongestant	P1P3	28	8.4	425	11.4	0.71	0.47, 1.06	0.81	0.54, 1.22
		P4P6	23	7.0	434	11.6	0.57	0.37, 0.88	0.65	0.42, 1.01
		P7P9	22	6.7	315	8.7	0.75	0.48, 1.18	0.83	0.52, 1.31
Tertiary amines	Analgesic	P1P3	12	3.8	57	1.7	**2.26**	**1.20, 4.26**	**2.40**	**1.26, 4.59**
		P4P6	13	4.1	81	2.4	1.73	0.95, 3.14	1.80	0.97, 3.31
		P7P9	8	2.5	77	2.3	1.12	0.53, 2.34	1.19	0.56, 2.52
	Analgesic opioid	P1P3	11	3.5	53	1.6	**2.23**	**1.15, 4.32**	**2.36**	**1.20, 4.64**
		P4P6	13	4.1	78	2.3	1.79	0.98, 3.26	**1.89**	**1.02, 3.49**
		P7P9	8	2.5	76	2.3	1.13	0.54, 2.37	1.21	0.57, 2.55

Table 2.6 Continued.

Type of drug exposure	Drug indication/ pharmacologic class[b]	Timing of drug exposure	Cases		Controls		Unadjusted OR[c]	95% CI	Adjusted OR[c,d]	95% CI
			No.	%	No.	%				
		P4P6	12	3.8	125	3.7	1.03	0.56, 1.89	1.08	0.58, 2.02
		P7P9	8	2.5	81	2.4	1.06	0.51, 2.22	1.05	0.49, 2.25
	Anti-emetic phenothiazine	P1P3	14	4.4	180	5.2	0.84	0.48, 1.46	0.91	0.51, 1.62
		P4P6	11	3.5	107	3.1	1.11	0.59, 2.08	1.13	0.58, 2.17
		P7P9	7	2.2	76	2.3	0.99	0.45, 2.17	0.97	0.43, 2.17
	Antihistamine	P1P3	20	6.1	271	7.6	0.79	0.50, 1.27	0.89	0.55, 1.44
		P4P6	26	7.8	219	6.2	1.28	0.84, 1.95	1.39	0.90, 2.14
		P7P9	24	7.3	172	5.0	1.50	0.96, 2.34	**1.63**	**1.03, 2.56**
	Cough	P1P3	8	2.5	114	3.3	0.75	0.36, 1.56	0.79	0.38, 1.64
		P4P6	7	2.2	85	2.5	0.89	0.41, 1.93	0.83	0.38, 1.84
		P7P9	7	2.2	63	1.8	1.19	0.54, 2.63	1.32	0.59, 2.94
	Anti-infective	P1P3	26	7.8	295	8.2	0.95	0.62, 1.44	1.00	0.65, 1.54
		P4P6	27	8.1	282	7.9	1.03	0.68, 1.55	1.07	0.70, 1.63
		P7P9	28	8.4	238	6.7	1.26	0.84, 1.90	1.31	0.86, 1.99
Amides	Anti-infective beta lactam	P1P3	22	6.7	264	7.4	0.90	0.57, 1.41	0.95	0.60, 1.51
		P4P6	23	7.0	255	7.2	0.97	0.62, 1.51	1.00	0.64, 1.57
		P7P9	26	7.8	215	6.1	1.30	0.85, 1.99	1.33	0.86, 2.05
	Cardiovascular	P1P3	2	0.6	9	0.3	2.39	0.51, 11.1	2.28	0.47, 11.0

Table 2.6 Continued.

Type of drug exposure	Drug indication/ pharmacologic class[b]	Timing of drug exposure	Cases		Controls		Unadjusted OR[c]	95% CI	Adjusted OR[c,d]	95% CI
			No.	%	No.	%				
Amides	Cardiovascular	P4P6	3	1.0	8	0.2	**4.03**	**1.06, 15.3**	3.80	0.97, 14.8
		P7P9	3	1.0	8	0.2	**4.03**	**1.06, 15.3**	3.80	0.98, 14.8

Abbreviations: OR, odds ratio; CI, confidence interval; P1P3- first trimester; P4P6- second trimester; P7P9- third trimester

[a]Data was restricted to participants with gestational ages between 37 and 41 weeks.

[b]Results are presented for drugs with at least 5 exposed cases and controls except for cardiovascular amides <5 cases were exposed to these drugs.

[c]Crude and adjusted odds ratios include only cases and controls with complete information for drug exposures and covariates.

[d]Adjusted for maternal race/ethnicity, education, smoking, chronic hypertension, and study center.

Discussion

In this population-based case-control study, we found that maternal exposure to nitrosatable drugs and its specific functional groups (secondary amines, tertiary amines, or amides) any time during pregnancy was not associated with SGA. However, when nitrosatable drugs exposure was examined by each trimester of pregnancy, exposure to drugs classified as nitrosatable amides during the third trimester of pregnancy was significantly associated with SGA. Higher ORs were noted for SGA in relation to amide drug exposure during the eighth and ninth month of pregnancy. Furthermore, these associations were stronger when analyses were restricted to full term case- and control-infants, and exposure to amides during the sixth month of pregnancy was also associated with SGA in full term births.

The findings of our study were similar to those observed in a Collaborative Perinatal Project that examined the association between nitrosatable drug use during pregnancy and adverse pregnancy outcomes.[93] Women who took nitrosatable drugs anytime during pregnancy were less likely to have low birthweight (<2000 grams) infants compared to women who did not report taking these drugs (OR 0.67 [95% CI 0.55, 0.82]). However, nitrosatable drugs were not classified by functional groups (i.e., secondary and tertiary amines, or amides) and its relation with SGA was not examined. In the present study, we found that the odds of having an infant with birth weight <2000 grams was lower in women exposed to nitrosatable drugs anytime during pregnancy

(OR 0.83 [95% CI 0.52, 1.31]) compared to women with no nitrosatable drug exposure anytime during pregnancy; but the 95% CI included the null.

Within the functional groups of nitrosatable drugs categorized by indication of use, we found null associations between several drugs indications and SGA except for a few notable exceptions. Prenatal use of antihistamines, classified as tertiary amines, during the third trimester of pregnancy was positively associated with full term SGA births in this study population. Oral antihistamines such as diphenhydramine, promethazine, meclizine, and cyclizine are generally used to control nausea and vomiting during pregnancy. Using data from the Swedish Medical Birth Registry, Asker and colleagues noted that women who reported any antiemetic drug use during pregnancy had a lower odds of having SGA births (OR 0.90 [95% CI 0.82, 0.99]).[152] However, promethazine use, the second most commonly reported drug, during pregnancy slightly elevated the risk of SGA (OR 1.07 [95% CI 1.06, 1.39]). The better infant outcome observed with any antiemetic use might not be attributable to the treatment itself but probably to a well-functioning placenta; placental hormones are proposed to play a role in the etiology of nausea and vomiting.

SGA was also associated with prenatal use of analgesic opioids, classified as tertiary amines, during the first or second trimester of pregnancy. Several studies have shown intrauterine growth restriction (IUGR) to be a common feature in pregnancies of opioid dependent mothers. Methadone, a synthetic opioid, generally used for the management of maternal opioid

dependency during pregnancy has been associated with SGA. Wouldes & Woodward found a significant linear association between maternal exposure to methadone and birthweight (p-value 0.001), especially with higher dose of methadone (>58 mg/day).[142] Also, women exposed to increasing doses of methadone were more likely to have a SGA infant (p-value 0.005). In a retrospective cohort study, Liu et al. noted that the risk of SGA was four times higher ([95%CI 1.70, 7.14]) among opioid dependent mothers who received methadone treatment compared to mothers not dependent on opioids.[143] Although prenatal exposure to methadone increased the risk of SGA, the beneficial effects of minimizing illicit drug use and maintaining the pregnancy may outweigh the risks of neonatal outcomes associated with methadone treatment.

With respect to nitrosatable secondary amines, maternal exposure to terbutaline, an asthma tocolytic agent, during the second trimester of pregnancy was significantly associated with SGA. Terbutaline, a β_2 adrenergic agonist, is used to relieve acute symptoms of asthma and is generally considered safe during pregnancy. In a cohort of 2,123 asthmatic patients recruited from 16 centers of the National Institute Child Health and Human Development Maternal Fetal Medicines Unit Network, Schatz et al. observed no significant difference in the incidence of SGA between women exposed to inhaled β_2 agonists during pregnancy and those not exposed to these drugs.[109] Bakhireva et al also found no increased risk of SGA births among women with asthma who reported

prenatal use of β_2 agonists compared to non-asthmatic controls (OR 0.57 [95% CI 0.16, 2.12]).[106]

Drugs indicated for cardiovascular conditions (classified as nitrosatable amides, or secondary or tertiary amines) were associated with SGA. However, very few women were exposed to these drugs in this study population. Several studies indicated that prenatal use of beta blockers, which are widely used for the treatment of hypertension and cardiac disorders, may increase the risk of SGA births. In a Danish birth cohort of 974,805 births between 1995 and 2008, Petersen et al. found that exposure to beta blockers during pregnancy was significantly associated with SGA (OR 1.97 [95% CI 1.75, 2.23]).[119] Specifically, labetalol, a beta blocker considered to be safe during pregnancy was strongly associated with SGA (OR 2.02 [95% CI 1.72, 2.37]). Furthermore, Lydakis et al. noted a significantly higher proportion of SGA babies (70%; p-value 0.01) among women who received atenolol during early pregnancy (<20 weeks) compared to 39.3% of women exposed later during pregnancy (>30 weeks).[123] These findings were corroborated by Bayliss et al., who found that the risk of SGA was higher among women exposed to atenolol <15 weeks of gestation (OR 2.81 [95% 1.27, 6.24]) than those not exposed to these drugs.[121]

The present study had several strengths. The study population was well representative of the US population. Cogswell et al. found that NBDPS control participants were generally characteristic of their base populations with respect to age, previous live births, and smoking.[179] Slight differences were noted by

maternal race/ethnicity, education, and infant characteristics if controls were selected from hospitals than from birth certificates. Also, since the study population did not include infants with any major birth defects, it allows a clearer interpretation of the study results because SGA and other neonatal outcomes are more commonly observed in infants with congenital malformations.[180, 181]

The findings of our study should be interpreted in context of the following limitations. First, a potential recall bias of drug exposures during pregnancy due to maternal self-report of drug use. Because the study utilized exposure data for all NBDPS controls who had births without congenital malformations, the possibility of recall bias is less likely. Previous studies have found little evidence for differential recall of drugs classified as nitrosatable in the present study. No difference in recall was observed for several drugs that have nitrosatable components including analgesics, antibiotics, and antinauseants between women with normal or adverse pregnancy outcomes.[197] The sensitivity and specificity of maternal recall for antibiotic, antinauseant, and any drug use during pregnancy was noted to be similar between low birth weight and control-infants.[196] To reduce recall bias, the NBDPS utilizes a two-level approach to assess drug usage by asking participants about drugs by indication of use and medication names. This approach has shown to be more accurate than an open ended questionnaire.[198, 199] In the NBDPS, women were asked about medication use during pregnancy and the drugs were later classified into secondary amines, tertiary amines, and amides depending on their nitrosatability. Because, women

were not aware of the nitrosatable components in the drugs, recall bias would have been unlikely. However, it is possible that some type of drugs within the various categories of nitrosatable drugs might have been recalled differently between case and control-women.

Another limitation pertains to missing exposure data due to lack of published literature on nitrosatability of the drugs. An extensive review of nitrosatable medicinal compounds published by Brambilla & Martelli[89] and McKean Cowdin et al.[185]; and published literature on nitrosatable drugs was used to classify drugs based on nitrosatable functional groups present. However, components of some of the drugs reported might not have been tested for nitrosatability, and results of such tests may not be published.

Information on dose of the drugs was not collected in the NBDPS interview. However, drug dose may not correspond to the quantity of N-nitroso compounds formed since conversion of nitrosatable drugs to N-nitroso compounds is dependent on presence and concentration of nitrite in stomach, gastric pH, reaction time, and other catalysts and inhibitors of the reaction.[89, 200]

In this study, multiple logistic regression models were fit to examine the relation between nitrosatable drug exposure during pregnancy and SGA birth. A total of 104 statistical tests were conducted to assess this association (52 for SGA and 52 for full term SGA births). Six statistically significant associations (adjusted OR >1.0 and 95% excluded the null value) were observed whereas only five tests would be expected by chance alone. However, given the number

of regression models analyzed and few significant results observed, there is insufficient evidence to conclude that prenatal use of nitrosatable drugs may be associated with SGA.

Overall, prenatal use of drugs that have nitrosatable components was not found to be associated with SGA except maternal use of nitrosatable amides during the third trimester of pregnancy slightly elevated the risk of SGA births. Our study focused only on the relation of nitrosatable drugs with SGA; however, nitrosatable amines or amides can react with nitrosating agents such as nitrite to form N nitroso compounds. Evidence from animal models suggested that combined exposure to nitrite and nitrosatable compound might influence fetal growth.[92] Future studies should explore the possible role of nitrosatable drugs and dietary nitrites on risk of SGA births.

3. DIETARY NITRATES AND NITRITES, NITROSATABLE DRUGS, AND SMALL-FOR-GESTATIONAL-AGE BIRTHS

Overview

Exposure to nitrates and nitrites from diet is fairly common and contributes a significant portion of daily nitrite exposure. Nitrites can react with amine or amide containing nitrosatable drugs to form N-nitroso compounds. Experimental data suggest that exposure to these compounds might reduce fetal birthweight. Using data from the National Birth Defects Prevention Study (NBDPS) control-mothers, we examined the association between maternal dietary intake of nitrates and nitrites and small-for-gestational-age (SGA) among 526 case-mothers of infants with birthweight <10th percentile and 5970 control-mothers during 1997-2005. The daily intake of nitrates, nitrites, and total nitrites was estimated from information collected using a 58-item food frequency questionnaire. Tertiles of each dietary component were calculated based on the control-mothers' distribution. Higher estimated intake of dietary nitrates, nitrites, and total nitrites was significantly associated with SGA, but none of these associations were significant after adjustment for covariates. Dietary nitrites modified the associations between nitrosatable drugs and SGA but lower odds of SGA were observed among women with higher nitrite intake. Strong associations were noted between exposure to nitrosatable amides during the third trimester of pregnancy and SGA among mothers with the lowest estimated

intake of dietary nitrite (OR 1.91 [95% CI 1.00, 3.66]) and animal nitrite (OR 2.31 [95% CI 1.22, 4.35]). Maternal exposure to dietary nitrite and total nitrite did not appear to be associated with SGA, nor did higher nitrite intake strengthen the association between nitrosatable drugs and SGA.

Background

Fetal growth is an important indicator of the individual's chance of survival and health in later life.[206] Babies born small-for-gestational-age (SGA) are at increased risk of development of poor cognitive and neurological disorders, and chronic diseases in adulthood.[3-6] The causes of SGA are not well established. Evidence suggests that reduced substrate delivery to the fetus caused by placental insufficiency or poor maternal nutrition may play a role in the etiology of small-for-gestational-age (SGA).[7] Several aspects of maternal diet have been hypothesized to influence fetal growth including dietary patterns;[8-10] vegetable and fruit consumption;[11-13] nutrient intake;[12, 14-16] and vitamin C and folic acid supplementation.[14, 15, 18] Kwong et al. demonstrated that changes in maternal diet during pregnancy can cause structural and functional abnormalities in organ and tissue development, and reduce fetal growth rate in animal models.[17] Small variations in maternal dietary patterns during early pregnancy and adoption of a healthy and nutrient rich diet have been associated with reduced risk of SGA birth.[8, 9, 18] However, limited studies have examined the effect of dietary nitrates and nitrites on risk of SGA birth.

Exposure to nitrates and nitrites can occur from diet, drinking water, certain medications, and environmental or occupational sources of which dietary consumption accounts for a significant portion of daily nitrite exposure. Nitrates are usually present in vegetables and root crops; and cured meat, baked goods, and cereals are common sources of dietary nitrite.[189] Approximately 5% of the nitrates ingested are converted to nitrites in the saliva and a portion of the nitrite is reduced to nitric oxide in the acidic environment of the stomach.[20, 87, 189] Nitric oxide is also produced endogenously by endothelial cells from L-arginine and it plays an important role in preimplantation embryo development, and placental vascular development.[22] Higher nitric oxide levels have been shown to arrest the cell cycle division that could result in apoptosis but their effect on fetal growth is unknown. In an experimental study in pregnant rats, an increase in serum nitrate/nitrite concentrations, following administration of diethylenetriamine-nitric oxide, decreased both placental and fetal weight compared to the control rats.[82] Studies have detected elevated nitric oxide levels in placenta and umbilical cord blood of pregnancies with intrauterine growth restriction compared to normal pregnancies.[83, 84] Additionally, Hata et al. noted maternal and fetal nitrate and nitrite concentrations to be significantly lower among women with SGA infants than those with appropriate for gestational age births suggesting that nitric oxide synthesis may be decreased in pregnancies with SGA infants.[85]

Ingested nitrates and nitrites could also react with nitrosatable amine (secondary or tertiary amine) or amide containing drugs to form N-nitroso

compounds.[24] Endogenous formation of these compounds contributes to 40 to 75% of human exposure.[88] Several N-nitroso compounds have been shown to cause congenital malformations in animal models;[90, 202-204] however very few studies have examined the relation between exposure to N-nitroso compounds and SGA. Only one experimental study observed fetal weight to be significantly reduced when pregnant mice were exposed to nitrite in combination with ethylenethiourea (a nitrosatable compound) but no effect was observed when exposed separately suggesting that N-nitroso compounds, formed from combination of nitrosatable compound and nitrite, might influence fetal growth.[92]

In this study, we examined 1) the relation between maternal consumption of dietary nitrates and nitrites and SGA births; and 2) whether higher intake of dietary nitrates and nitrites modified the association between nitrosatable drugs use during pregnancy and SGA births.

Methods

We used data from control participants of the National Birth Defects Prevention Study (NBDPS), a large population-based case control study of birth defects in the United States. Since the study's inception in 1997, ten Centers for Birth Defects Research and Prevention (CBDRP) including Arkansas, California, Georgia, Iowa, Massachusetts, New York, and Texas (from 1998 to present); New Jersey (from 1998 to 2002); and North Carolina and Utah (from 2003 to present) have participated in the study.

Study population

We focused on NBDPS control mothers who had live births without major birth defects with estimated dates of delivery (EDDs) between October 1, 1997, and December 31, 2005; and were residents of one of the geographic areas covered by the CBDRP population registries. They were randomly sampled from either birth certificates (Arkansas, for EDDs after 2000; Georgia, for EDDs after 2000; Iowa; Massachusetts; New Jersey; North Carolina; and Utah) or hospital records (Arkansas, for EDDs prior to 2001; California; Georgia, for EDDs prior to 2001; New York; and Texas).[179] States that selected controls from hospitals utilized a systematic random sampling scheme so that infants selected were in proportion to the number of births at each hospital in the geographic area.[178] The controls-infants were ineligible if they were stillborn, had a major birth defect, were adopted or in foster care, had a deceased mother, or were born outside the study area. We included data only on mothers who delivered singleton births since multiple births have been identified as a major risk factor for SGA. The institutional review boards in each state and the Center for Disease Control and Prevention approved the NBDPS protocol, and the institutional review board of Texas A&M University approved this study.

Case and control definition

Cases were defined as infants with birthweight less than the 10th percentile for given gestational age, gender, and race/ethnicity. Infants with birthweight at or greater than 10th percentile were identified as controls. The US

singleton birthweight percentiles for gestational age by maternal race, parity, and infant gender, published by Overpeck et al.[182] and Zhang & Bowes,[183] were used for classification of SGA. Infant with gestational ages less than 20 weeks or more than 44 weeks were excluded. In NBDPS, information on gestational age and birthweight at delivery were obtained from medical records or birth certificates of the participants. If not available, the following criteria was used for calculation of gestational age: 1) estimated due date reported by mother in the interview; 2) ultrasound <14 weeks; 3) last menstrual period; 4) ultrasound >14 weeks; or 5) standard neonatal exam.

Data collection

The NBDPS utilized a standard procedure for contacting the mothers and enrolling them in the study. The interviews were targeted for completion within six months of EDD until 24 months post-delivery. In the original study, women were not interviewed until six weeks after the EDD or actual date of delivery to reduce recall bias between women with preterm and full term births. After oral informed consent was obtained, the interviews were conducted either in English or Spanish by trained female interviewers using a computer-assisted telephone interview. It took approximately 1-1½ hours to complete and covered topics regarding maternal health (including medications taken); diet (food consumption in the year before pregnancy); work history; demographic characteristics; and water use.[178] A pregnancy calendar was provided that helped mothers to recollect exposures by date, month, or trimester of pregnancy. Data from the

NBDPS with EDDs from 1997-2005 had a total of 6807 (66.2%) control mothers who participated in the interview and the median length of time from EDD to interview was 7.7 months.

Estimation of dietary intake of nitrates and nitrites

Information on foods consumed during the year prior to conception was collected using a 58-item food frequency questionnaire (FFQ) which was adapted from the Short Willett Food Frequency Questionnaire. Women were also asked about consumption of breakfast cereals from three months prior to conception through the end of pregnancy. Additional questions regarding certain region-specific food items such as avocados, raw chili peppers, salsa, refried beans, and tortillas were added to the questionnaire to incorporate the diverse diet of the NBDPS population.

The maternal dietary intake of nitrates and nitrites was estimated using a multi-step procedure developed by Griesenbeck and colleagues.[188] Briefly, the nitrate and nitrite values for each food item or group listed in the FFQ were estimated based on extensive review of published literature. Weighted means of the nitrates and nitrites values in mg/100g were calculated for each food item and multiplied by the serving size in grams. The mean nitrate and nitrite estimates in each serving size were multiplied by the number of servings per month, summed across all food items, and divided by 30 to calculate the average daily intake of nitrate and nitrite in mg for each participant. Since approximately 5% of the nitrates ingested are converted endogenously to

nitrites, the estimate for total dietary nitrite was calculated as [dietary nitrite + (0.05 X nitrate intake)] based on the method suggested by Choi et al.[189] Tertiles of nitrates, nitrites from either plant or animal sources, and total nitrites were generated based on the distribution of control women. Complete data for total nitrite and covariates was available for 94.9% and 95.9% of the case and control participants.

Classification of nitrosatable drugs

As a part of the NBDPS interview, women were questioned about prescription and non-prescription drugs taken (medication name), the corresponding dates and frequency of use from three months prior to conception to the date of birth of index pregnancy. Information was collected on drugs used for specific illness and diseases (e.g., asthma, diabetes, hypertension etc.), and about specific products (e.g., ampicillin, phenytoin, metoprolol). The Slone Epidemiology Center Drug Dictionary was used to link the reported drugs to their active ingredients.[184]

Detailed methods used to classify drugs with respect to nitrosatability, functional groups, and indications were described in previous publications.[25, 174] The methodology used for classification included: 1) active ingredients for all orally administered drugs, and orally inhaled medications were identified; 2) these active ingredients were cross referenced with a comprehensive list of nitrosatable medicinal compounds published by Brambilla & Martelli[89] and McKean Cowdin et al.;[185] 3) identified nitrosatable compounds were categorized

based on the presence of amine (secondary or tertiary) and amide functional groups; and further 5) classified by the drug's primary indication (e.g., antihistamine, antiepileptic) and pharmacologic class (e.g., opioid, macrolide). We focused on maternal exposure to nitrosatable drugs anytime or by each trimester (first, second, or third) of pregnancy. For each nitrosatable drug, information on whether the drug was taken and frequency of use was used to estimate exposure for each month and trimester of pregnancy. Complete information on nitrosatable drug use anytime during pregnancy was available for 99% and 98.5% of case and control participants.

Statistical analyses

Unconditional binary logistic regression was used to analyze the association of maternal dietary intake of nitrates, nitrites (plant or animal source), and total nitrites with SGA. Tertiles of nitrates, nitrites, and total nitrites were calculated based on the distributions among control mothers; and the lowest tertile of each dietary component was used as the referent group. All dietary analyses were conducted using Stata 11[205] and were restricted to women who had daily caloric intake between 500-5000 kcal. These limits were recommended by Willett[190] and have been previously used by dietary studies and those utilizing the NBDPS database.[174, 191]

Covariates included in the logistic models were selected based on their association with SGA and maternal risk factors associated with nitrosatable drugs use from previous literature. Maternal race/ethnicity, education, and study

center were important predictors of nitrosatable drug use among control participants of NBDPS as noted in a previous publication.[25] Non-significant covariates as well as those that did not change the odds ratio by 10 percent or more were eliminated from the final model using forward selection. The following covariates were included in the final model: maternal race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, Asian/Pacific Islander, other), maternal education (<12 years, 12 years, 13-15 years, >15 years), study center, maternal smoking (yes, no), and chronic hypertension prior to pregnancy (yes, no). Only participants with complete information available for all covariates included in the final logistic models were used for both crude and adjusted analyses.

We examined whether dietary nitrite intake modified the association between nitrosatable drug exposure and SGA. Stratum specific odds ratio and 95% confidence intervals were estimated for each tertile of dietary nitrites and total nitrites with adjustment for aforementioned covariates and total energy intake. Additive and multiplicative interaction was assessed for the associations of SGA with nitrosatable drugs by dietary nitrites and total nitrites. We tested for additive interaction using a statistical program developed by Andersson & colleagues that estimated measures of relative excess risk due to interaction (RERI) and attributable proportion due to interaction (AP).[193] If either or both measures differed from zero and their 95% confidence intervals excluded 0, significant additive interaction was considered present. To assess multiplicative

interaction, the product term of nitrosatable drug functional groups with dietary nitrite and total nitrite were included in the logistic models and was considered significant if the p-value was less than 0.05.

We also examined the association between dietary intake of nitrites and total nitrites and SGA among a subgroup of full term case and control infants with gestational ages restricted between 37 and 41 weeks. Since the associations observed between nitrosatable drugs and SGA stratified by dietary nitrites and total nitrites were similar for analyses including all study participants and with restriction to full term case and control infants, we present findings only for full term SGA infants.

Results

A total of 526 infants classified as SGA and 5,970 controls-infants with an EDD from 1997-2005 participated in the NBDPS. The median length of time from EDD to interview was eight months for both case and control participants. Compared to control mothers, case mothers were more likely to be Hispanic or Asian/Pacific Islander, had less than 12 years of education, delivered between 20-24 years of age, and reported smoking during pregnancy (Table 3.1). A higher percentage of case mothers (10.5%) had body mass index (BMI) less than 18.5 kg/m^3 than control mothers (4.8%). Case mothers were also more likely to have a lower gestational weight gain of <25lbs (34.0%) compared to control mothers (25.0%). The distributions of maternal characteristics including race/ethnicity, education, age at delivery, study center, BMI, smoking, gender,

Table 3.1 Selected Maternal Characteristics of Small-For-Gestational-Age Infants (Cases) and Controls in the National Birth Defects Prevention Study, 1997-2005

Characteristics of Participants	Controls n=5,970		Cases n=526		OR	95% CI
	No.	%	No.	%		
Race-ethnicity*						
Non-Hispanic white	3573	59.9	289	54.9	1.00	Referent
Non-Hispanic black	685	11.5	44	8.4	0.79	0.57, 1.10
Hispanic	1310	21.9	141	26.8	**1.33**	**1.08, 1.64**
Asian/Pacific Islander	165	2.8	27	5.1	**2.02**	**1.32, 3.09**
All others	237	4.0	25	4.8	1.30	0.85, 2.00
Missing	0	0	0	0	-	-
Education (years)*						
>15	1882	31.5	114	21.7	1.00	Referent
13-15	1597	26.8	142	27.0	**1.47**	**1.14, 1.89**
12	1448	24.3	140	26.6	**1.60**	**1.23, 2.06**
<12	960	16.1	126	24.0	**2.17**	**1.66, 2.82**
Missing	83	1.4	4	0.8	-	-
Age at delivery (years)*						
<18	219	3.7	22	4.2	0.91	0.57, 1.46
18-19	420	7.0	42	8.0	0.91	0.64, 1.30
20-24	1356	22.7	149	28.3	1.00	Referent
25-29	1601	26.8	133	25.3	0.76	0.59, 0.97
30-34	1569	26.3	98	18.6	0.57	0.44, 0.74
>34	805	13.5	82	15.6	0.93	0.70, 1.23
Study center*						
Arkansas	747	12.5	78	14.8	1.00	Referent
California	760	12.7	62	11.8	0.78	0.55, 1.11
Georgia	674	11.3	55	10.5	0.78	0.54, 1.12
Iowa	742	12.4	62	11.8	0.80	0.56, 1.13
Massachusetts	492	8.2	49	9.3	0.95	0.66, 1.39
North Carolina	539	9.0	46	8.8	0.82	0.56, 1.20
New Jersey	675	11.3	82	15.6	1.16	0.84, 1.61
New York	648	10.9	44	8.4	0.65	0.44, 0.95
Texas	360	6.0	23	4.4	0.61	0.38, 0.99
Utah	333	5.6	25	4.8	0.72	0.45, 1.15
Body mass index (kg/m^2)*						
<18.5	289	4.8	55	10.5	**2.08**	**1.52, 2.84**
18.5–24.9	3205	53.7	293	55.7	1.00	Referent
25.0–29.9	1308	21.9	95	18.1	0.79	0.62, 1.01
>29.9	948	15.9	54	10.3	**0.62**	**0.46, 0.84**
Missing	220	3.7	29	5.5	-	-
Smoking*						
No	4809	81.5	392	75.0	1.00	Referent
Yes	1094	18.5	131	25.0	**1.47**	**1.19, 1.81**
Missing	0	0	0	0	-	-
Gender*						
Male	2992	50.1	297	56.5	1.00	Referent
Female	2978	49.9	229	43.5	**1.29**	**1.08, 1.54**
Missing	0	0	0	0	-	-

Table 3.1 Continued.

Characteristics of Participants	Controls n=5,970		Cases n=526		OR	95% CI
	No.	%	No.	%		
Parity						
Nulliparous	2392	40.1	204	38.8	1.00	Referent
Primiparous	1993	33.4	187	35.6	1.10	0.89, 1.35
Multiparous	1585	26.6	135	25.7	1.00	0.80, 1.25
Gestational weight gain*						
<25lbs	1492	25.0	179	34.0	**1.27**	**1.03, 1.57**
25-35lbs	2088	35.0	197	37.5	1.00	Referent
>35lbs	2154	36.1	127	24.1	0.62	0.50, 0.79
Missing	236	4.0	23	4.4	-	-

Abbreviations: OR, odds ratio; CI, confidence interval.

* $p < 0.05$; statistically significant difference in distribution between cases and controls participants

and gestational weight gain were significantly different between case and control mothers.

Estimated maternal dietary intake of nitrates, nitrites, total nitrites, and animal nitrites was significantly associated with SGA, but none of these associations remained significant after adjustment for covariates (Table 3.2). Compared to the lowest tertile of dietary nitrate intake (<31.43 mg/day), women who consumed nitrates of more than 51.74 mg/day were slightly more likely to have SGA births (adjusted odds ratio (aOR) 1.06 [95% CI 0.82, 1.39]). Higher estimated intake of animal source of dietary nitrite (>1.20 mg/day) (aOR 1.05 [95% CI 0.81, 1.35], respectively) was associated with SGA but the 95% confidence interval included the null. Restriction of analyses to full term case and control infants did not materially change the adjusted ORs for SGA with

Table 3.2 Maternal Dietary Intake of Nitrates and Nitrites and Risk of Small-For-Gestational-Age Births, National Birth Defects Prevention Study, 1997-2005

Dietary contaminant	Tertiles mg/day	Cases		Controls		Unadjusted OR[a]	95% CI	Adjusted OR[a,b]	95% CI
		No.	%	No.	%				
Nitrates	<31.43	146	29.3	1909	33.3	1.00	Referent	1.00	Referent
	31.43-51.74	165	33.1	1909	33.3	1.13	0.90, 1.42	1.05	0.82, 1.33
	>51.74	188	37.6	1909	33.3	**1.29**	**1.03, 1.61**	1.06	0.82, 1.39
Nitrites	<1.28	144	28.8	1910	33.3	1.00	Referent	1.00	Referent
	1.28-1.90	155	31.0	1914	33.4	1.07	0.85, 1.36	0.95	0.75, 1.22
	>1.90	201	40.2	1910	33.3	**1.40**	**1.12, 1.74**	0.94	0.70, 1.25
Total Nitrites	<3.02	144	28.9	1906	33.3	1.00	Referent	1.00	Referent
	3.02-4.54	161	32.3	1914	33.4	1.11	0.88, 1.41	1.00	0.79, 1.28
	>4.54	194	38.8	1907	33.3	**1.35**	**1.08, 1.69**	0.99	0.75, 1.31
Animal Nitrites	<0.74	150	29.9	1918	33.3	1.00	Referent	1.00	Referent
	0.74-1.20	150	29.9	1926	33.4	1.00	0.79, 1.26	0.94	0.74, 1.20
	>1.20	202	40.2	1916	33.3	**1.35**	**1.08, 1.68**	1.05	0.81, 1.35
Plant Nitrites	<0.46	157	31.2	1919	33.3	1.00	Referent	1.00	Referent
	0.46-0.70	153	30.4	1918	33.3	0.98	0.77, 1.23	0.86	0.68, 1.10
	>0.70	194	38.4	1915	33.3	1.24	0.99, 1.54	0.82	0.61, 1.10

Abbreviations: OR, odds ratio; CI, confidence interval.

[a]Crude and adjusted odds ratios include only cases and controls with complete information for drug exposures and covariates, and whose daily caloric intake was between 500 and 5000 kcal.

[b]Adjusted for maternal race/ethnicity, education, smoking, chronic hypertension, caloric intake, and study center.

Table 3.3. Maternal Dietary Intake of Nitrates and Nitrites and Risk of Full Term[a] Small-For-Gestational Age Births, National Birth Defects Prevention Study, 1997-2005

Dietary contaminant	Tertiles mg/day	Cases		Controls		Unadjusted OR[b]	95% CI	Adjusted OR[b,c]	95% CI
		No.	%	No.	%				
Nitrates	<31.43	137	29.9	1719	33.2	1.00	Referent	1.00	Referent
	31.43-51.74	153	33.3	1723	33.2	1.11	0.88, 1.42	1.04	0.81, 1.33
	>51.74	169	36.8	1741	33.6	1.22	0.96, 1.54	1.01	0.77, 1.34
Nitrites	<1.28	131	28.5	1731	33.4	1.00	Referent	1.00	Referent
	1.28-1.90	142	30.9	1728	33.3	1.09	0.85, 1.39	0.96	0.75, 1.24
	>1.90	187	40.6	1730	33.3	**1.43**	**1.13, 1.80**	0.97	0.72, 1.31
Total nitrites	<3.02	135	29.4	1717	33.1	1.00	Referent	1.00	Referent
	3.02-4.54	150	32.7	1736	33.5	1.10	0.86, 1.40	0.99	0.76, 1.27
	>4.54	174	37.9	1730	33.4	**1.28**	**1.01, 1.62**	0.93	0.70, 1.25
Animal nitrites	<0.74	136	29.4	1740	33.4	1.00	Referent	1.00	Referent
	0.74-1.20	138	29.8	1739	33.4	1.02	0.79, 1.30	0.95	0.74, 1.23
	>1.20	189	40.8	1732	33.2	**1.40**	**1.11, 1.76**	1.09	0.83, 1.41
Plant nitrites	<0.46	146	31.5	1729	33.2	1.00	Referent	1.00	Referent
	0.46-0.70	142	30.7	1731	33.3	0.97	0.76, 1.24	0.86	0.67, 1.11
	>0.70	175	37.8	1746	33.5	1.19	0.94, 1.49	0.77	0.57, 1.05

Abbreviations: OR, odds ratio; CI, confidence interval.

[a]Data was restricted to participants with gestational ages between 37 and 41 weeks.

[b]Crude and adjusted odds ratios include only cases and controls with complete information for drug exposures and covariates, and whose daily caloric intake was between 500 and 5000 kcal.

[c]Adjusted for maternal race/ethnicity, education, smoking, chronic hypertension, caloric intake, and study center.

dietary nitrates and animal nitrites in the highest tertile (versus the lowest tertile) (aOR 1.01 [95% CI 0.77, 1.34] and aOR 1.09 [95% CI 0.83, 1.41], respectively) (Table 3.3).

Overall, higher dietary consumption of nitrites or total nitrites in conjunction with nitrosatable drug use during pregnancy did not increase the risk of SGA births. However, a higher odds of SGA births was observed among women with the lowest estimated intake of dietary nitrites and total nitrites in relation to nitrosatable drugs exposure during pregnancy. Exposure to drugs classified as nitrosatable amides during the third trimester of pregnancy was significantly associated with SGA in women with lowest estimated intake of dietary nitrites (<1.28 mg/day) (aOR 1.91 [95% CI 1.00, 3.66]) (Table 3.4). Stronger associations were also noted between amide drug exposure during the eighth and ninth month of pregnancy and SGA for the lowest tertile of dietary nitrite intake (aOR 3.15 [95% CI 1.53, 6.51] and (aOR 2.43 [95% CI 1.02, 5.77], respectively) (data not shown). Exposure to amides during the 6th month of pregnancy was also significantly associated with SGA in women with the lowest estimate intake of dietary nitrite (aOR 2.39 [95% CI 1.13, 5.50]). A pattern of decreasing odds ratios was observed between nitrosatable drug exposure and SGA by increasing levels of total nitrite (sum of dietary nitrite and 5% dietary nitrate) (Table 3.5). A significant association was observed between amide exposure during the 8th month of pregnancy and SGA for the lowest tertile of total nitrite intake (aOR 2.91 [95% CI 1.45, 5.84]) (data not shown).

Table 3.4 Maternal Exposure to Nitrosatable Drugs by Each Trimester of Pregnancy and Small-For-Gestational Age[a] Births by Estimated Dietary Intake of Nitrites, National Birth Defects Prevention Study, 1997-2005

Dietary intake mg/day	Timing of exposure	Type of drug exposure	Cases No.	Cases %[b]	Controls No.	Controls %[b]	Unadjusted OR[c]	95% CI	Adjusted OR[c,d]	95% CI
Nitrites		No nitrosatable drug								
< 1.28	First	exposure	78	70.9	1057	71.7	Referent	Referent	Referent	Referent
	trimester	Secondary amines	18	18.8	220	17.2	1.11	0.65, 1.89	1.16	0.67, 2.02
		Tertiary amines	13	14.3	223	17.4	0.79	0.43, 1.45	0.82	0.44, 1.54
		Amides	10	11.4	123	10.4	1.10	0.56, 2.18	1.15	0.57, 2.33
		No nitrosatable drug								
1.28-1.90		exposure	86	71.1	1027	71.2	Referent	Referent	Referent	Referent
		Secondary amines	17	16.5	217	17.4	0.94	0.54, 1.61	1.10	0.63, 1.93
		Tertiary amines	13	13.1	204	16.6	0.76	0.42, 1.39	0.85	0.45, 1.60
		Amides	10	10.4	137	11.8	0.87	0.44, 1.72	0.90	0.45, 1.82
		No nitrosatable drug								
> 1.90		exposure	127	78.4	1114	71.4	Referent	Referent	Referent	Referent
		Secondary amines	17	11.8	206	15.6	0.72	0.43, 1.23	0.76	0.44, 1.32
		Tertiary amines	18	12.4	219	16.4	0.72	0.43, 1.21	0.76	0.44, 1.32
		Amides	12	8.6	131	10.5	0.80	0.43, 1.49	0.81	0.42, 1.54
Nitrites		No nitrosatable drug								
< 1.28	Second	exposure	78	69.6	1057	73.9	Referent	Referent	Referent	Referent
	trimester	Secondary amines	15	16.1	221	17.3	0.92	0.52, 1.63	1.03	0.57, 1.85
		Tertiary amines	13	14.3	173	14.1	1.02	0.55, 1.87	1.04	0.55, 1.96
		Amides	13	14.3	109	9.4	1.62	0.87, 3.00	1.60	0.84, 3.05
		No nitrosatable drug								
1.28-1.90		exposure	86	74.1	1027	72.4	Referent	Referent	Referent	Referent
		Secondary amines	17	16.5	235	18.6	0.86	0.50, 1.48	0.98	0.56, 1.72
		Tertiary amines	8	8.5	180	14.9	0.53	0.25, 1.11	0.56	0.26, 1.20
		Amides	11	11.3	116	10.2	1.13	0.59, 2.18	1.26	0.64, 2.46

Table 3.4 Continued.

Dietary intake mg/day	Timing of exposure	Type of drug exposure	Cases No.	Cases %[b]	Controls No.	Controls %[b]	Unadjusted OR[c]	95% CI	Adjusted OR[c,d]	95% CI
		No nitrosatable drug exposure	127	77.4	1114	75.8	Referent	Referent	Referent	Referent
> 1.90		Secondary amines	20	13.6	204	15.5	0.86	0.52, 1.41	0.90	0.53, 1.52
		Tertiary amines	25	16.5	169	13.2	1.30	0.82, 2.05	1.33	0.81, 2.17
		Amides	12	8.6	117	9.5	0.90	0.48, 1.68	0.90	0.47, 1.72
Nitrites		No nitrosatable drug exposure								
< 1.28	Third trimester		78	69.6	1057	75.2	Referent	Referent	Referent	Referent
		Secondary amines	17	17.9	205	16.2	1.12	0.65, 1.94	1.23	0.70, 2.17
		Tertiary amines	17	17.9	159	13.1	1.45	0.84, 2.51	1.49	0.84, 2.63
		Amides	13	14.3	91	7.9	**1.94**	**1.04, 3.62**	**1.91**	**1.00, 3.66**
		No nitrosatable drug exposure	86	74.8	1027	74.5	Referent	Referent	Referent	Referent
1.28-1.90		Secondary amines	16	15.7	218	17.5	0.88	0.50, 1.52	1.02	0.57, 1.80
		Tertiary amines	9	9.5	148	12.6	0.73	0.36, 1.47	0.75	0.36, 1.57
		Amides	8	8.5	92	8.2	1.04	0.49, 2.21	1.14	0.53, 2.48
		No nitrosatable drug exposure	127	79.4	1114	78.5	Referent	Referent	Referent	Referent
>1.90		Secondary amines	15	10.6	174	13.5	0.76	0.43, 1.32	0.82	0.46, 1.46
		Tertiary amines	17	11.8	129	10.4	1.16	0.67, 1.98	1.23	0.69, 2.17
		Amides	15	10.6	105	8.6	1.25	0.71, 2.22	1.31	0.72, 2.40

Abbreviations: OR, odds ratio; CI, confidence interval.

[a]Data was restricted to participants with gestational ages between 37 and 41 weeks.

[b]Percentages for no nitrosatable drug exposure are based on total participants with complete information whereas percentages for secondary or tertiary amines or amides includes complete information for the given drug group and excludes other nitrosatable groups in the denominator.

[c]Crude and adjusted odds ratios include only cases and controls with complete information for drug exposures and covariates, and whose daily caloric intake was between 500 and 5000 kcal.

[d]Adjusted for maternal race/ethnicity, education, smoking, chronic hypertension, caloric intake, and study center.

Table 3.5 Maternal Exposure to Nitrosatable Drugs by Each Trimester of Pregnancy and Small-For-Gestational-Age[a] Births by Estimated Dietary Intake of Total Nitrites, National Birth Defects Prevention Study, 1997-2005

Dietary intake mg/day	Timing of exposure	Type of drug exposure	Cases		Controls		Unadjusted OR[c]	95% CI	Adjusted OR[c,d]	95% CI
			No.	%[b]	No.	%[b]				
Total Nitrites		No nitrosatable drug								
< 3.02	First	exposure	83	75.5	1040	71.4	Referent	Referent	Referent	Referent
	trimester	Secondary amines	14	14.4	219	17.4	0.80	0.45, 1.44	0.89	0.49, 1.62
		Tertiary amines	13	13.5	215	17.1	0.76	0.41, 1.38	0.81	0.44, 1.52
		Amides	7	7.8	121	10.4	0.72	0.33, 1.60	0.78	0.34, 1.76
		No nitrosatable drug								
3.02-4.54		exposure	85	66.4	1025	70.1	Referent	Referent	Referent	Referent
		Secondary amines	23	21.3	234	18.6	1.19	0.73, 1.92	1.30	0.79, 2.15
		Tertiary amines	20	19.1	233	18.5	1.04	0.62, 1.72	1.12	0.66, 1.91
		Amides	9	9.6	150	12.8	0.72	0.36, 1.47	0.76	0.37, 1.57
		No nitrosatable drug								
>4.54		exposure	122	79.2	1130	75.8	Referent	Referent	Referent	Referent
		Secondary amines	15	11.0	189	14.3	0.74	0.42, 1.28	0.79	0.44, 1.41
		Tertiary amines	11	8.3	196	14.8	0.52	0.28, 0.98	0.56	0.29, 1.10
		Amides	16	11.6	120	9.6	1.23	0.71, 2.15	1.29	0.72, 2.33
Total Nitrites		No nitrosatable drug								
< 3.02	Second	exposure	83	72.8	1040	73.6	Referent	Referent	Referent	Referent
	trimester	Secondary amines	13	13.5	221	17.5	0.74	0.40, 1.35	0.84	0.45, 1.57
		Tertiary amines	13	13.5	163	13.6	1.00	0.54, 1.83	1.01	0.53, 1.90
		Amides	11	11.7	107	9.3	1.29	0.67, 2.49	1.31	0.66, 2.59
		No nitrosatable drug								
3.02-4.54		exposure	85	69.1	1025	71.8	Referent	Referent	Referent	Referent
		Secondary amines	23	21.3	234	18.6	1.19	0.73, 1.92	1.29	0.78, 2.12
		Tertiary amines	15	15.0	193	15.9	0.94	0.53, 1.66	1.01	0.56, 1.82
		Amides	13	13.3	134	11.6	1.17	0.64, 2.15	1.23	0.65, 2.31

Table 3.5 Continued.

Dietary intake mg/day	Timing of exposure	Type of drug exposure	Cases No.	Cases %[b]	Controls No.	Controls %[b]	Unadjusted OR[c]	95% CI	Adjusted OR[c,d]	95% CI
>4.54		No nitrosatable drug exposure	122	79.2	1130	76.7	Referent	Referent	Referent	Referent
		Secondary amines	16	11.6	205	15.4	0.72	0.42, 1.24	0.81	0.46, 1.43
		Tertiary amines	18	12.9	166	12.8	1.00	0.60, 1.69	1.06	0.61, 1.84
		Amides	12	9.0	101	8.2	1.10	0.59, 2.06	1.09	0.56, 2.12
Total Nitrites		No nitrosatable drug exposure								
< 3.02	Third trimester		83	71.6	1040	75.3	Referent	Referent	Referent	Referent
		Secondary amines	14	14.4	205	16.5	0.86	0.48, 1.54	0.96	0.53, 1.77
		Tertiary amines	16	16.2	147	12.4	1.36	0.78, 2.39	1.42	0.79, 2.55
		Amides	14	14.4	95	8.4	**1.85**	**1.01, 3.38**	1.83	0.98, 3.42
3.02-4.54		No nitrosatable drug exposure	85	71.4	1025	73.9	Referent	Referent	Referent	Referent
		Secondary amines	18	17.5	219	17.6	0.99	0.58, 1.68	1.12	0.65, 1.93
		Tertiary amines	14	14.1	167	14.0	1.01	0.56, 1.82	1.05	0.57, 1.95
		Amides	10	10.5	94	8.4	1.28	0.64, 2.55	1.32	0.65, 2.68
>4.54		No nitrosatable drug exposure	122	80.8	1130	78.9	Referent	Referent	Referent	Referent
		Secondary amines	16	11.6	173	13.3	0.86	0.50, 1.48	0.95	0.54, 1.68
		Tertiary amines	13	9.6	122	9.7	0.99	0.54, 1.80	1.04	0.55, 1.96
		Amides	12	9.0	99	8.1	1.12	0.60, 2.10	1.15	0.60, 2.21

Abbreviations: OR, odds ratio; CI, confidence interval.

[a]Data was restricted to participants with gestational ages between 37 and 41 weeks.

[b]Percentages for no nitrosatable drug exposure are based on total participants with complete information whereas percentages for secondary or tertiary amines or amides includes complete information for the given drug group and excludes other nitrosatable groups in the denominator.

[c]Crude and adjusted odds ratios include only cases and controls with complete information for drug exposures and covariates, and whose daily caloric intake was between 500 and 5000 kcal.

[d]Adjusted for maternal race/ethnicity, education, smoking, chronic hypertension, caloric intake, and study center.

Higher ORs were noted between nitrosatable drugs exposure and SGA among women with lower estimated dietary intake of animal nitrite, especially with exposure to nitrosatable amides during the second and third trimester of pregnancy (aOR 2.11 [95% CI 1.12, 3.97) and (aOR 2.31 [95% CI 1.22, 4.35], respectively) (Table 3.6). When exposure to amides was examined by each month of pregnancy, stronger associations were observed between SGA and amide drug exposures during the 5^{th} (aOR = 3.17), 6^{th} (aOR= 3.34), 8^{th} (aOR = 3.40) and 9^{th} (aOR = 3.11) months of pregnancy for the lowest tertile of animal nitrite intake (<0.74 mg/day). Although some fruits and vegetables are known to contain vitamin C that may inhibit nitrosation, maternal intake of plant nitrites had minimal effect on the association between nitrosatable drugs and SGA (Table 3.7). But exposure to amides during the 5^{th} month of pregnancy was associated with SGA in women with estimated daily intake of 0.46-0.70 mg (middle tertile) of plant nitrite (aOR 2.19 [95% CI 1.10, 4.37]) (data not shown).

Discussion

In this population based case-control study, we found that maternal exposure to higher dietary intake of nitrates, nitrites, and total nitrites were not significant risk factors for SGA. Moreover, women with the lowest estimated intake of nitrite and total nitrites were more likely to have SGA offspring if they reported exposure to drugs classified as nitrosatable amides during the third trimester of pregnancy. A lower intake of animal source of dietary nitrites in conjunction with exposure to amide drugs was significantly associated with SGA,

Table 3.6 Maternal Exposure to Nitrosatable Drugs by Each Trimester of Pregnancy and Small-For-Gestational Age[a] Births by Estimated Dietary Intake of Animal Nitrites, National Birth Defects Prevention Study, 1997-2005

			Cases		Controls					
Dietary intake mg/day	Timing of exposure	Type of drug exposure	No.	%[b]	No.	%[b]	Unadjusted OR[c]	95% CI	Adjusted OR[c,d]	95% CI
Animal Nitrites		No nitrosatable drug								
< 0.74	First	exposure	83	69.8	1113	74.3	Referent	Referent	Referent	Referent
	trimester	Secondary amines	19	18.6	209	15.8	1.22	0.72, 2.05	1.37	0.79, 2.36
		Tertiary amines	15	15.3	197	15.0	1.02	0.58, 1.81	1.16	0.64, 2.13
		Amides	13	13.5	112	9.1	1.56	0.84, 2.88	1.90	1.00, 3.62
		No nitrosatable drug								
0.74-1.20		exposure	89	77.4	1048	71.7	Referent	Referent	Referent	Referent
		Secondary amines	14	13.6	218	17.2	0.76	0.42, 1.35	0.88	0.48, 1.62
		Tertiary amines	11	11.0	206	16.4	0.63	0.33, 1.20	0.73	0.37, 1.44
		Amides	5	5.3	136	11.5	0.43	0.17, 1.08	0.46	0.18, 1.18
		No nitrosatable drug								
>1.20		exposure	121	74.7	1053	71.4	Referent	Referent	Referent	Referent
		Secondary amines	19	13.6	219	17.2	0.76	0.46, 1.25	0.82	0.48, 1.39
		Tertiary amines	19	13.6	243	18.8	0.68	0.41, 1.13	0.74	0.43, 1.26
		Amides	14	10.4	145	12.1	0.84	0.47, 1.50	0.88	0.48, 1.60
Animal Nitrites		No nitrosatable drug								
< 0.74	Second	exposure	83	72.2	1113	75.6	Referent	Referent	Referent	Referent
	trimester	Secondary amines	14	14.4	218	16.4	0.86	0.48, 1.55	1.02	0.56, 1.88
		Tertiary amines	11	11.7	164	12.8	0.90	0.47, 1.72	1.03	0.52, 2.02
		Amides	14	14.4	104	8.6	1.81	0.99, 3.29	**2.11**	**1.12, 3.97**
		No nitrosatable drug								
0.74-1.20		exposure	89	76.1	1048	74.0	Referent	Referent	Referent	Referent
		Secondary amines	16	15.2	219	17.3	0.86	0.50, 1.49	0.97	0.54, 1.72
		Tertiary amines	11	11.0	168	13.8	0.77	0.40, 1.47	0.83	0.42, 1.63
		Amides	8	8.3	112	9.7	0.84	0.40, 1.78	0.90	0.41, 1.97

Table 3.6 Continued.

Dietary intake mg/day	Timing of exposure	Type of drug exposure	Cases		Controls		Unadjusted OR[c]	95% CI	Adjusted OR[c,d]	95% CI
			No.	%[b]	No.	%[b]				
>1.20		No nitrosatable drug exposure	121	74.2	1053	72.6	Referent	Referent	Referent	Referent
		Secondary amines	22	15.4	226	17.7	0.85	0.53, 1.36	0.93	0.56, 1.53
		Tertiary amines	25	17.1	194	15.6	1.12	0.71, 1.77	1.18	0.73, 1.90
		Amides	14	10.4	128	10.8	0.95	0.53, 1.70	0.96	0.52, 1.75
Animal Nitrites		No nitrosatable drug								
< 0.74	Third	exposure	83	72.2	1113	77.0	Referent	Referent	Referent	Referent
	trimester	Secondary amines	17	17.0	200	15.2	1.14	0.66, 1.96	1.33	0.75, 2.34
		Tertiary amines	14	14.4	148	11.7	1.27	0.70, 2.29	1.39	0.75, 2.59
		Amides	14	14.4	90	7.5	**2.09**	**1.14, 3.82**	**2.31**	**1.22, 4.35**
0.74-1.20		No nitrosatable drug exposure	89	74.2	1048	75.5	Referent	Referent	Referent	Referent
		Secondary amines	17	16.0	207	16.5	0.97	0.56, 1.66	1.01	0.58, 1.78
		Tertiary amines	13	12.8	137	11.6	1.12	0.61, 2.05	1.18	0.62, 2.25
		Amides	7	7.3	88	7.8	0.94	0.42, 2.08	0.99	0.43, 2.25
>1.20		No nitrosatable drug exposure	121	78.1	1053	76.0	Referent	Referent	Referent	Referent
		Secondary amines	14	10.4	190	15.3	0.64	0.36, 1.14	0.71	0.39, 1.29
		Tertiary amines	16	11.7	151	12.5	0.92	0.53, 1.60	0.97	0.55, 1.71
		Amides	16	11.7	110	9.5	1.27	0.73, 2.21	1.33	0.74, 2.40

Abbreviations: OR, odds ratio; CI, confidence interval.

[a]Data was restricted to participants with gestational ages between 37 and 41 weeks.

[b]Percentages for no nitrosatable drug exposure are based on total participants with complete information whereas percentages for secondary or tertiary amines or amides includes complete information for the given drug group and excludes other nitrosatable groups in the denominator.

[c]Crude and adjusted odds ratios include only cases and controls with complete information for drug exposures and covariates, and whose daily caloric intake was between 500 and 5000 kcal.

[d]Adjusted for maternal race/ethnicity, education, smoking, chronic hypertension, caloric intake, and center.

Table 3.7 Maternal Exposure to Nitrosatable Drugs by Each Trimester of Pregnancy and Small-For-Gestational-Age[a] Births by Estimated Dietary Intake of Plant Nitrites, National Birth Defects Prevention Study, 1997-2005

Dietary intake mg/day	Timing of exposure	Type of drug exposure	Cases		Controls		Unadjusted OR[c]	95% CI	Adjusted OR[c,d]	95% CI
			No.	%[b]	No.	%[b]				
Plant Nitrites		No nitrosatable drug								
< 0.46	First	exposure	84	71.2	1031	70.3	Referent	Referent	Referent	Referent
	trimester	Secondary amines	15	15.2	224	17.9	0.82	0.47, 1.45	0.86	0.48, 1.54
		Tertiary amines	15	15.2	218	17.5	0.84	0.48, 1.49	0.90	0.50, 1.62
		Amides	10	10.6	145	12.3	0.85	0.43, 1.67	0.85	0.42, 1.70
		No nitrosatable drug								
0.46-0.70		exposure	86	69.9	974	67.9	Referent	Referent	Referent	Referent
		Secondary amines	21	19.6	248	20.3	0.96	0.58, 1.58	0.96	0.58, 1.61
		Tertiary amines	17	16.5	261	21.1	0.74	0.43, 1.26	0.72	0.41, 1.27
		Amides	13	13.1	137	12.3	1.07	0.58, 1.98	1.17	0.62, 2.21
		No nitrosatable drug								
>0.70		exposure	122	78.7	1207	78.8	Referent	Referent	Referent	Referent
		Secondary amines	16	11.6	173	12.5	0.92	0.53, 1.58	1.13	0.64, 2.02
		Tertiary amines	13	9.6	168	12.2	0.77	0.42, 1.39	0.97	0.51, 1.84
		Amides	11	8.3	110	8.4	0.99	0.52, 1.89	1.10	0.56, 2.18
Plant Nitrites		No nitrosatable drug								
< 0.46	Second	exposure	84	67.7	1031	72.4	Referent	Referent	Referent	Referent
	trimester	Secondary amines	18	17.7	231	18.3	0.96	0.56, 1.62	1.02	0.59, 1.77
		Tertiary amines	15	15.2	175	14.5	1.05	0.59, 1.86	1.10	0.61, 1.98
		Amides	12	12.5	121	10.5	1.22	0.65, 2.29	1.20	0.62, 2.29
		No nitrosatable drug								
0.46-0.70		exposure	86	71.1	974	69.3	Referent	Referent	Referent	Referent
		Secondary amines	19	18.1	261	21.1	0.82	0.49, 1.38	0.85	0.50, 1.45
		Tertiary amines	16	15.7	212	17.9	0.85	0.49, 1.49	0.80	0.45, 1.43
		Amides	15	14.9	122	11.1	1.39	0.78, 2.49	1.46	0.79, 2.69

Table 3.7 Continued.

Dietary intake mg/day	Timing of exposure	Type of drug exposure	Cases No.	Cases %[b]	Controls No.	Controls %[b]	Unadjusted OR[c]	95% CI	Adjusted OR[c,d]	95% CI
>0.70		No nitrosatable drug exposure	122	81.3	1207	80.3	Referent	Referent	Referent	Referent
		Secondary amines	15	11.0	170	12.4	0.87	0.50, 1.53	1.11	0.61, 2.01
		Tertiary amines	15	11.0	135	10.1	1.10	0.62, 1.93	1.37	0.75, 2.53
		Amides	11	8.3	100	7.7	1.09	0.57, 2.08	1.16	0.59, 2.28
Plant Nitrites		No nitrosatable drug								
< 0.46	Third	exposure	84	70.0	1031	74.6	Referent	Referent	Referent	Referent
	trimester	Secondary amines	17	16.8	225	17.9	0.93	0.54, 1.59	0.99	0.57, 1.73
		Tertiary amines	18	17.7	152	12.9	1.45	0.84, 2.49	1.51	0.87, 2.64
		Amides	11	11.6	88	7.9	1.53	0.79, 2.98	1.64	0.82, 3.26
0.46-0.70		No nitrosatable drug exposure	86	73.5	974	71.3	Referent	Referent	Referent	Referent
		Secondary amines	15	14.9	224	18.7	0.76	0.43, 1.34	0.74	0.41, 1.33
		Tertiary amines	11	11.3	180	15.6	0.69	0.36, 1.32	0.66	0.34, 1.29
		Amides	14	14.0	114	10.5	1.39	0.77, 2.53	1.45	0.77, 2.74
>0.70		No nitrosatable drug exposure	122	79.7	1207	82.2	Referent	Referent	Referent	Referent
		Secondary amines	16	11.6	149	11.0	1.06	0.61, 1.84	1.36	0.76, 2.42
		Tertiary amines	14	10.3	104	7.9	1.33	0.74, 2.40	1.71	0.90, 3.23
		Amides	13	9.6	86	6.7	1.50	0.81, 2.76	1.53	0.80, 2.92

Abbreviations: OR, odds ratio; CI, confidence interval.

[a]Data was restricted to participants with gestational ages between 37 and 41 weeks.

[b]Percentages for no nitrosatable drug exposure are based on total participants with complete information whereas percentages for secondary or tertiary amines or amides includes complete information for the given drug group and excludes other nitrosatable groups in the denominator.

[c]Crude and adjusted odds ratios include only cases and controls with complete information for drug exposures and covariates, and whose daily caloric intake was between 500 and 5000 kcal.

[d]Adjusted for maternal race/ethnicity, education, smoking, chronic hypertension, caloric intake, and study center.

but consumption of plant source of nitrites had no effect on risk of SGA births.

Several studies have examined the individual associations of SGA with dietary patterns, single food nutrients, and vegetable and fruit consumption during pregnancy. Using data from a Danish National Birth Cohort of 44,612 women, Knudsen and colleagues found that women classified in the health conscious group characterized by higher intake of vegetables, fruits, fish, and poultry, had lower odds of having a SGA infant (OR 0.74 [95% CI 0.64, 0.86]) than women with high intake of red and processed meat.[8] These findings were corroborated by Thompson & colleagues who reported that women who adopted a traditional diet during early pregnancy, that included mostly fruits and vegetables, were less likely to have SGA births (OR 0.86 [95% CI 0.75, 0.99]).[9] Furthermore, a retrospective cohort study conducted in Spain observed that women in the lowest quintile of vegetable intake during the third trimester of pregnancy had higher odds of having a SGA infant (OR 2.1 [95% CI 1.0, 4.7]).[13] Vegetables, particularly spinach contain the highest amount of nitrates per serving (189 mg/serving).[188] In the present study, we found a negative association between higher dietary intake of plant source of nitrites and SGA but the 95% confidence interval included the null (OR 0.82 [95% CI 0.61, 1.10]).

Experimental studies have demonstrated that drugs containing nitrosatable groups such as amines or amides can react with nitrosating agents such as nitrite under simulated gastric conditions to form N-nitroso compounds or nitrosamines. Choi et al. reported that if the nitrite concentration is high, the

formation of N nitroso compounds would be to a greater extent.[189] Only one published study examined the association between N-nitroso compounds and adverse pregnancy outcomes. In pregnant mice exposed to nitrite in combination with ethylenethiourea (a nitrosatable compound), fetal weight was significantly reduced when both compounds were administered together, but no effect was observed when given separately.[92] In this study population, a decreasing trend was noted in odds of SGA with exposure to nitrosatable drugs by increasing levels of nitrite intake. We observed strongest associations between nitrosatable amides and SGA among participants with the lowest intake of dietary nitrites and total nitrites.

To our knowledge this is one of the first studies that examined the relation of maternal dietary consumption of nitrates and nitrites with SGA. One of the strengths of the study was the large study population from multiple regions in the United States. Cogswell et al. found that NBDPS control participants were generally characteristic of their base populations with respect to age, previous live births, and smoking.[179] Slight differences were noted by maternal race/ethnicity, education, and infant characteristics if the control participants were selected from hospitals compared to birth certificates. Also, since the study population did not include infants with any major birth defects, it allows a clearer interpretation of the study results because SGA and other neonatal outcomes are more commonly observed in infants with congenital malformations. Lastly, the nitrate and nitrite estimates used in the study were based on extensive

review by Griesenbeck and colleagues of all published literature regarding nitrates and nitrite content of food items listed in the Willett FFQ.

The study had several limitations. In the NBDPS, participants were interviewed about the frequency of foods consumed a year prior to conception which could be subject to participants' recall and may have resulted in misclassification of foods consumed during pregnancy. However, the misclassification might be non-differential with respect to the outcome (SGA) as the same period of dietary assessment was used for all NBDPS participants. Further, studies have indicated that consumption of vegetables and meats, major source of nitrates and nitrites, respectively, measured by consecutive 7-day dietary records did not significantly differ before and during pregnancy, and strong correlations have been reported between vegetable intake at the beginning and end of pregnancy.[13, 195]

Data collected from the FFQ could possibly have some degree of measurement error due to participants' self-report of dietary consumption. However, when the dietary data used in this study were examined for measurement error using the Simulation Extrapolation (SIMEX) algorithm, Huber et al. found no difference in statistical significance or effect size for models in which the amount of error varied from 0 to 60% additional variance in increment of 10%.[207] Furthermore, studies of validity and reproducibility of the original Willett FFQ indicate that the dietary questionnaire provides useful information about women's nutrient intake over a one year period compared to four one

week diet records.[186] The assessment of dietary intake of nutrients using the Willett FFQ was also comparable to nutrient intakes estimated using 24 hour recalls.[187]

Another limitation pertains to the potential maternal recall bias of drug exposures during pregnancy. Because the study utilized exposure data for all NBDPS controls who had births without congenital malformations, the possibility of recall bias is less likely. Previous studies have found little evidence for differential recall of drugs classified as nitrosatable in the present study. No difference in recall was observed for several drugs that have nitrosatable drug components including analgesics, antibiotics, and antinauseants between women with normal or adverse pregnancy outcomes.[197] The sensitivity and specificity of maternal recall for antibiotic, antinauseant, and any drug use during pregnancy was noted to be similar between low birth weight and control-infants.[196] To reduce recall bias, the NBDPS utilizes a two-level approach to assess drug usage by asking participants about drugs by indication of use and medication names. This approach has shown to be more accurate than an open ended questionnaire.[198, 199] In the NBDPS, women were asked about medication use during pregnancy and the drugs were later classified into secondary amines, tertiary amines, and amides depending on their nitrosatability. Because, women were not aware of the nitrosatable drug components in the drugs, recall bias would have been less likely. However, it is possible that some type of drugs

within the various categories of nitrosatable drugs might have been recalled differently between case and control-women.

Exposure to some drugs may have been missed due to lack of published information on their nitrosatability. An extensive review of nitrosatable medicinal compounds published by Brambilla & Martelli[89] and McKean Cowdin et al.[185]; and published literature on nitrosatable drugs was used to classify drugs based on nitrosatable functional groups present. However, components of some of the drugs reported might not have been tested for nitrosatability, and results of such tests may not be published.

Multiple analyses and comparisons were performed with respect to nitrosatable drug use stratified by dietary nitrite (plant and animal nitrite) and total nitrite. In study analyses, 108 logistic models were fit to assess the association between nitrosatable drugs and SGA by tertiles of dietary nitrite and total nitrite. Five associations would be expected by chance but only three adjusted odds ratios had 95% confidence intervals that excluded the null. To assess the interaction between nitrosatable drugs and dietary nitrites and total nitrites with SGA, 36 statistical tests were conducted. Two tests would be expected by chance but no significant interaction was observed.

In conclusion, findings from this study suggest that higher consumption of dietary nitrites and total nitrites during pregnancy may not increase the risk of SGA. We examined if higher intake of dietary nitrites and total nitrites strengthened the associations between nitrosatable drugs and SGA; but a

reverse trend was noted with modest associations observed for the highest tertile of dietary nitrites intake. Few significant results were found between nitrosatable amides and SGA for the lowest estimated intake of nitrite and animal source of nitrites. Although endogenous formation of N-nitroso compounds is one of the suggested mechanisms in the etiology of SGA, exposure to other environmental toxicants and other sources of nitrates such as drinking water might contribute to fetal growth restriction and their effect on the relation between dietary nitrates and SGA needs to be further examined.

4. PRENATAL EXPOSURE TO NITROSATABLE DRUGS, VITAMIN C, AND RISK OF SMALL-FOR-GESTATIONAL-AGE BIRTHS

Overview

Certain drugs, which contain secondary or tertiary amines or amides, can react with nitrite in the stomach to form N-nitroso compounds. These compounds have been associated with reduced birthweight in some animal models. Vitamin C is known nitrosation inhibitor. We examined the effect of vitamin C on the relation between maternal use of nitrosatable drugs during pregnancy and small-for-gestational-age (SGA) birth. Data were analyzed from control participants (mothers of babies without major birth defects) of the National Birth Defects Prevention Study that included 526 mothers who delivered infants with birthweight < 10^{th} percentile and 5,970 mothers of control infants (birthweight ≥ 10^{th} percentile for gestational age) born during 1997-2005. Maternal reports of vitamin C supplement use and data collected from a food frequency questionnaire was used to estimate daily intake of vitamin C. Daily use of supplements containing vitamin C presented inconsistent findings on the association between nitrosatable drugs and SGA. Higher intake of dietary vitamin C (≥85 mg/day) in conjunction with daily vitamin C supplementation slightly reduced the associations between SGA and secondary amines during the second trimester of pregnancy (adjusted odds ratio [aOR] 1.0 [95%

confidence interval [CI] 0.65, 1.6]) compared with <85mg of dietary vitamin C and less than daily use of vitamin C supplement (OR 4.0 [95% CI 1.5, 10.9]). Prenatal use of dietary and supplemental vitamin C may modify the association between SGA and selected nitrosatable drugs.

Background

Low birthweight (<2500 g) is an important determinant of infant mortality. In 2011, disorders related to low birthweight and short gestation were the second leading cause of all infant deaths in the United States.[208] However, low birth weight could represent both infants born prematurely and those with intrauterine growth restriction (IUGR). Small-for-gestational-age (SGA), usually defined as infants with birthweight less than 10th percentile for gestational age, is a commonly used proxy measure for assessment of IUGR. SGA infants are at increased risk of mortality during the first year of life, growth deficits and poor developmental outcomes in childhood, and chronic diseases in adulthood such as cardiovascular disease, insulin resistance, diabetes mellitus, dyslipidemia, and renal disease.[3-6] Size at birth is dependent on the fetal growth rate which is influenced by a wide range of factors such as maternal body mass index, gestational weight gain, smoking and maternal nutrition during pregnancy.[7, 11, 27-29, 209] In the Dutch Famine of 1994-1945, severe malnutrition of mothers during the third trimester of pregnancy was associated with reduced fetal and placental weight.[210] Although maternal undernutrition is uncommon in developed countries, the relative deficiency or lower levels of certain micronutrients such as

vitamin C might affect fetal growth. Vitamin C, a hydrophilic antioxidant, negates the effect of oxygen free radicals and it may help protect against cellular damage in the fetus.[211] But the role of vitamin C on risk of SGA births in women exposed to nitrosatable drugs during pregnancy is not known.

A variety of prescription and over-the-counter medications, which contain nitrosatable amines (secondary or tertiary amines) or amides, react with nitrosating agents such as nitrites in the acidic environment of the stomach to form N-nitroso compounds.[24] Endogenous formation of these compounds accounts for 40 to 75% of human exposure.[88] N-nitroso compounds have been found to be associated with limb malformations, oral clefts, and neural tube and craniofacial defects in animal models.[90, 202-204] However, relatively few studies have examined the relation between exposure to these compounds and birth outcomes. In pregnant mice exposed to ethylenethiourea (a nitrosatable compound) and nitrite, fetal weight was observed to be significantly reduced when both compounds were administered together but no effect was observed when given separately suggesting that N-nitroso compounds, formed from combination of nitrosatable compound and nitrite, might influence fetal growth.[92]

Vitamin C is a well-documented inhibitor of nitrosation. Studies have found vitamin C to reduce formation of N-nitroso compounds *in vivo* when administered with a nitrosatable compound. Mirvish et al. demonstrated that ascorbic acid inhibits *in vivo* nitrosation by rapid reduction of nitrite to nitrous oxide followed by production of dehydroascorbic acid.[172] In a clinical trial

conducted with human volunteers, concomitant administration of increasing doses of ascorbic acid (1.76-1000mg) with nitrate and nitrosatable precursor such as proline significantly reduced the excretion of N-nitroso compounds by 44% compared to combined exposures to nitrate and proline without ascorbic acid.[173] In our previous study, we found that relative to women with no vitamin C supplementation, daily use of vitamin C supplement in conjunction with first trimester exposure to nitrosatable drugs was associated with lower odds of several birth defects including transverse limb deficiency with secondary amines, cleft lip without cleft palate with tertiary amines, and several congenital heart defects with tertiary amines and amides.[175] No published study has examined the effect of vitamin C supplementation on SGA in relation to nitrosatable drug exposure during pregnancy.

In the present study, we examined the individual and joint effects of vitamin C supplementation and dietary vitamin C on the relation between SGA and maternal exposure to nitrosatable drugs (secondary or tertiary amines, or amides) during pregnancy.

Methods

To address the study objectives, data were used from control participants (mothers of babies without major birth defects) of the National Birth Defects Prevention Study (NBDPS), a large population-based case control study of birth defects in the United States that began in 1997. Ten Centers for Birth Defects Research and Prevention (CBDRP) including Arkansas, California, Georgia,

Iowa, Massachusetts, New York, and Texas (from 1998 to present); New Jersey (from 1998 to 2002); and North Carolina and Utah (from 2003 to present) have participated or are currently participating in the national study.

Study population

The present study included NBDPS control mothers who had live births without major birth defects with estimated dates of delivery (EDDs) between October 1, 1997, and December 31, 2005; and were residents of one of the geographic areas covered by the CBDRP population registries. These controls were randomly sampled from either birth certificates (Arkansas, for EDDs after 2000; Georgia, for EDDs after 2000; Iowa; Massachusetts; New Jersey; North Carolina; and Utah) or hospital records (Arkansas, for EDDs prior to 2001; California; Georgia, for EDDs prior to 2001; New York; and Texas).[179] For states that selected controls from hospitals, a systematic random sampling scheme was used so that infants selected were in proportion to the number of births at each hospital in the geographic area.[178] The controls-infants were not eligible if they were stillborn, had a major birth defect, were adopted or in foster care, had a deceased mother, or were born outside the study area. Data only on mothers who delivered singleton births were included in the study analyses since multiple births have been identified as a major risk factor for SGA. The institutional review boards in each state and the Center for Disease Control and Prevention approved the NBDPS protocol, and the institutional review board of Texas A&M University approved this study.

Case and control definition

Cases were defined as infants with birthweight less than the 10th percentile for given gestational age, gender, and race/ethnicity. Infants with birthweight at or greater than 10th percentile were identified as controls. Classification of SGA was based on the US singleton birthweight percentiles for gestational age by maternal race, parity, and infant gender, published by Overpeck et al.[182] and Zhang & Bowes.[183] Infant with gestational ages less than 20 weeks or more than 44 weeks were excluded. Data on birthweight in grams and gestational age in weeks were obtained from either medical records or birth certificates of the participants. If not available, the following criteria was used for calculation of gestational age: 1) estimated due date reported by mother in the interview; 2) ultrasound <14 weeks; 3) last menstrual period; 4) ultrasound >14 weeks; or 5) standard neonatal exam.

Data collection

In the NBDPS, women were interviewed via telephone by trained female interviewers in either English or Spanish using a structured questionnaire after oral consent was obtained.[178] The interview took approximately 1-1½ hours to complete and covered topics regarding maternal health (including medications taken); diet (food consumption in the year before pregnancy); work history; demographic characteristics; and water use. Interviews were targeted for completion within six months of EDD until 24 months post-delivery. Women were not interviewed until six weeks after the EDD or actual date of delivery to reduce

recall bias between women with preterm and full term births. Data from the NBDPS with EDDs from 1997-2005 had a total of 6807 (66.2%) control mothers who participated in the interview.

Classification of nitrosatable drugs

As part of the NBDPS interview, women were questioned about prescription and non-prescription drugs taken (medication name), the corresponding dates and frequency of use from three months prior to conception to the date of birth of index pregnancy. Women were also asked about drugs used for specific illness and diseases (e.g., asthma, diabetes, hypertension etc.), and specific products (e.g., ampicillin, phenytoin, metoprolol). The Slone Epidemiology Center Drug Dictionary was used to link the reported drugs to their active ingredients.[184]

Detailed methods used to classify drugs with respect to nitrosatability, functional groups, and indications were described in previous publications.[25, 174] The methodology used for classification included: 1) active ingredients for all orally administered drugs, and orally inhaled medications were identified; 2) these active ingredients were cross referenced with a comprehensive list of nitrosatable medicinal compounds published by Brambilla & Martelli[89] and McKean Cowdin et al.;[185] 3) identified nitrosatable compounds were categorized based on the presence of amine (secondary or tertiary) and amide functional groups; and further 5) classified by the drug's primary indication (e.g., antihistamine, antiepileptic) and pharmacologic class (e.g., opioid, macrolide).

We focused on maternal exposure to nitrosatable drugs during each trimester (first, second, or third) of pregnancy. Complete information on nitrosatable drug use anytime during pregnancy was available for 99% and 98.5% of case and control participants.

Assessment of vitamin C intake

The NBDPS collects information regarding the start and stop dates, duration, and frequency of vitamin supplement use (single, prenatal, and multivitamins) from three months prior to conception through the end of pregnancy. Vitamin C supplementation was categorized into none, less than daily, and daily depending on the frequency of intake. Women who reported using a daily vitamin C supplement during the first, second, or third trimester of pregnancy were classified as “daily” and those who no reported no vitamin C supplementation during the same period were classified as “none.” If women reported taking vitamin C supplement less than 90 days in a given trimester or less than every day in a given period, they were classified as “less than daily.” Due to small numbers, women with none and less than daily intake of vitamin C supplement were combined together. Complete information was available for 97.0% and 97.2% of case- and control-mothers with nitrosatable drug use anytime during pregnancy and vitamin C supplementation during the first and second trimester of pregnancy. Because of incomplete information available on vitamin C supplement use during the third trimester of pregnancy, findings are presented only for the first and second trimester of pregnancy.

Information on foods consumed during the year prior to conception was collected using a 58-item food frequency questionnaire (FFQ) which was adapted from the short Willett Food Frequency Questionnaire. Data was also obtained regarding consumption of cereal intake from three months prior to conception through the end of pregnancy. The Willett FFQ has been validated and reproduced in other studies and provides useful information about nutrient intake over a one-year period compared to 24-hour or one week dietary recalls.[186, 187] Daily intake of dietary vitamin C was calculated based on estimates from the NBDPS Nutrient Database for Standard Reference 19. Complete information on nitrosatable drug use anytime during pregnancy stratified by dietary vitamin C was available for 96.8% and 97.1% of the case and control participants.

Statistical analyses

Logistic regression was used to analyze the association between nitrosatable drug and SGA by supplemental and dietary intake of vitamin C. Nitrosatable drug exposure during the first, second, and third trimester of pregnancy was stratified by categories of vitamin C supplement use (<daily and daily). Women who reported no nitrosatable drug use anytime during pregnancy served as the referent group. We also examined the effect of dietary vitamin C (<85 mg/day or ≥85mg/day) in relation to nitrosatable drug use. The cut points were based on the recommended daily dietary vitamin C allowance for pregnant women over 18 years of age which corresponded to the 41st percentile for

control participants in this study. All dietary vitamin C analyses were restricted to women who had daily caloric intake between 500-5000 kcal. These limits were recommended by Willett[190] and have been previously used by dietary studies and those utilizing the NBDPS database.[174, 191]

We calculated daily intake of total vitamin C by combining estimates of dietary and supplemental vitamin C. Women with daily vitamin C supplementation and ≥85mg of dietary vitamin C were classified as "high/daily" and those with less than daily intake of vitamin C supplement and lower intake of dietary vitamin C were categorized as "low/none". We also examined the relation between nitrosatable drugs and SGA stratified by supplemental and dietary vitamin C among full term case and control infants with gestational ages restricted between 37 and 41 weeks. All analyses were performed using Stata 11.[205]

Covariates included in the logistic models were selected based on their association with SGA and maternal risk factors associated with nitrosatable drug use from previous literature. Maternal race/ethnicity, education, and study center were important predictors of nitrosatable drug use among control participants of NBDPS as noted in a previous publication.[25] Non-significant covariates as well as those that did not change the odds ratio by 10 percent or more were eliminated from the final model using forward selection. The following covariates were included in the final model: maternal race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, Asian/Pacific Islander, other), maternal education

(<12 years, 12 years, 13-15 years, >15 years), study center, maternal smoking (yes, no), and chronic hypertension prior to pregnancy (yes, no). Total caloric intake was included in the models of dietary vitamin C analyses in addition to the above mentioned covariates. Only participants with complete information available for all covariates included in the final logistic models were used for both crude and adjusted analyses.

Additive and multiplicative interaction was assessed for the associations of SGA with nitrosatable drugs by supplemental and dietary vitamin C. We tested for additive interaction using a statistical program developed by Andersson & colleagues that estimated measures of relative excess risk due to interaction (RERI) and attributable proportion due to interaction (AP).[193] If either or both measures differed from zero and their 95% confidence intervals excluded 0, significant additive interaction was considered present. To assess multiplicative interaction, the product term of nitrosatable drug functional groups with supplemental and dietary vitamin C were included in the logistic models and was considered significant if the p-value was less than 0.05.

Results

A total of 526 infants classified as SGA and 5,970 controls-infants (not classified as SGA) with an EDD from 1997-2005 participated in the NBDPS. The median length of time from EDD to interview was eight months for both case and control participants. Compared to control mothers, case mothers were more likely to be Hispanic or Asian/Pacific Islander, less educated, smokers, delivered

between 20-24 years of age, had a BMI less than 18.5 kg/m^3 and a gestational weight gain of <25lbs (Table 4.1). A lower proportion of case mothers than control mothers reported taking a daily supplement of vitamin C during the first and second trimester of pregnancy. The distributions of maternal characteristics including race/ethnicity, education, age at delivery, study center, BMI, smoking, gender, gestational weight gain, and vitamin C supplementation during the second trimester of pregnancy were significantly different between case and control mothers.

Daily use of supplements containing vitamin C presented discordant results on the association between nitrosatable drug use during pregnancy and SGA (Table 4.2). Among women who took a daily supplement of vitamin C, a higher odd of SGA was observed in conjunction with exposure to nitrosatable amides during the first trimester of pregnancy (OR 1.03 [95% CI 0.54, 1.96]) compared to women exposed to these drugs and less than daily intake of vitamin C supplement (OR 0.88 [95%CI 0.56, 1.38]). In contrast, exposure to secondary amines during the second trimester of pregnancy was strongly associated with SGA in women who did not take a vitamin C supplement on a daily basis (OR 1.63 [95% CI 0.88, 3.01] compared to women with daily vitamin C supplementation during the same time period (OR 0.83 [95% CI 0.59, 1.17]).

Higher intake of dietary vitamin C (≥85 mg/day) slightly reduced the associations between SGA and tertiary amines during the third trimester of pregnancy, and amide drug exposure during the second trimester of pregnancy

Table 4.1 Selected Maternal Characteristics of Small-For-Gestational-Age Infants (Cases) and Controls in the National Birth Defects Prevention Study, 1997-2005

Characteristics of Participants	Controls n=5,970		Cases n=526		OR	95% CI
	No.	%	No.	%		
Race-ethnicity*						
Non-Hispanic White	3573	59.9	289	54.9	1.00	Reference
Non-Hispanic Black	685	11.5	44	8.4	0.79	0.57, 1.10
Hispanic	1310	21.9	141	26.8	1.33	1.08, 1.64
Asian/Pacific Islander	165	2.8	27	5.1	**2.02**	**1.32, 3.09**
All others	237	4.0	25	4.8	1.30	0.85, 2.00
Missing	0	0	0	0	-	-
Education (years)*						
>15	1882	31.5	114	21.7	1.00	Reference
13-15	1597	26.8	142	27.0	**1.47**	**1.14, 1.89**
12	1448	24.3	140	26.6	**1.60**	**1.23, 2.06**
<12	960	16.1	126	24.0	**2.17**	**1.66, 2.82**
Missing	83	1.4	4	0.8	-	-
Age at delivery (years)*						
<18	219	3.7	22	4.2	0.91	0.57, 1.46
18-19	420	7.0	42	8.0	0.91	0.64, 1.30
20-24	1356	22.7	149	28.3	1.00	Reference
25-29	1601	26.8	133	25.3	0.76	0.59, 0.97
30-34	1569	26.3	98	18.6	0.57	0.44, 0.74
>34	805	13.5	82	15.6	0.93	0.70, 1.23
Study center*						
Arkansas	747	12.5	78	14.8	1.00	Reference
California	760	12.7	62	11.8	0.78	0.55, 1.11
Georgia	674	11.3	55	10.5	0.78	0.54, 1.12
Iowa	742	12.4	62	11.8	0.80	0.56, 1.13
Massachusetts	492	8.2	49	9.3	0.95	0.66, 1.39
North Carolina	539	9.0	46	8.8	0.82	0.56, 1.20
New Jersey	675	11.3	82	15.6	1.16	0.84, 1.61
New York	648	10.9	44	8.4	0.65	0.44, 0.95
Texas	360	6.0	23	4.4	0.61	0.38, 0.99
Utah	333	5.6	25	4.8	0.72	0.45, 1.15
Body mass index (kg/m^2)*						
<18.5	289	4.8	55	10.5	**2.08**	**1.52, 2.84**
18.5–24.9	3205	53.7	293	55.7	1.00	Reference
25.0–29.9	1308	21.9	95	18.1	0.79	0.62, 1.01
>29.9	948	15.9	54	10.3	0.62	0.46, 0.84
Missing	220	3.7	29	5.5	-	-
Smoking*						
No	4809	81.5	392	75.0	1.00	Reference
Yes	1094	18.5	131	25.0	**1.47**	**1.19, 1.81**
Missing	0	0	0	0	-	-
Gender*						
Male	2992	50.1	297	56.5	1.00	Reference
Female	2978	49.9	229	43.5	**1.29**	**1.08, 1.54**
Missing	0	0	0	0	-	-
Parity						
Nulliparous	2392	40.1	204	38.8	1.00	Reference

Table 4.1 Continued.

Characteristics of Participants		Controls n=5,970		Cases n=526		OR	95% CI
		No.	%	No.	%		
Primiparous		1993	33.4	187	35.6	1.10	0.89, 1.35
Multiparous		1585	26.6	135	25.7	1.00	0.80, 1.25
Gestational weight gain*							
<25lbs		1492	25.0	179	34.0	**1.27**	**1.03, 1.57**
25-35lbs		2088	35.0	197	37.5	1.00	Reference
>35lbs		2154	36.1	127	24.1	0.62	0.50, 0.79
Missing		236	4.0	23	4.4	-	-
Vitamin C supplement use							
First trimester*	<daily	3988	67.9	372	72.2	1.23	1.00, 1.50
	daily	1882	32.1	143	27.8	1.00	Reference
Second trimester*	<daily	1075	18.3	126	24.4	**1.44**	**1.17, 1.78**
	daily	4796	81.7	390	75.6	1.00	Reference
Dietary vitamin C							
<85 mg/day		2432	41.0	201	38.2	0.89	0.74, 1.07
≥85 mg/day		3507	59.0	325	61.8	1.00	Referent

Abbreviations: OR, odds ratio; CI, confidence interval.

* $p < 0.05$; statistically significant difference in distribution between cases and controls participants.

Table 4.2 Effects of Maternal Nitrosatable Drug Exposures by Each Trimester of Pregnancy on Small-For-Gestational-Age Births Stratified by Vitamin C Supplementation, National Birth Defects Prevention Study, 1997-2005

Frequency of vitamin C supplement	Timing of exposure[a]	Type of drug exposure	Cases		Controls		Unadjusted		Adjusted	
			No.	%[b]	No.	%[b]	OR[c]	95% CI	OR[c,d]	95% CI
<Daily	P1P3	No nitrosatable drug exposure	243	76.7	2495	73.9	1.00	Referent	1.00	Referent
		Secondary amines	36	12.9	457	15.5	0.81	0.56, 1.16	0.87	0.60, 1.27
		Tertiary amines	32	11.6	474	16.0	0.69	0.47, 1.02	0.77	0.51, 1.14
		Amides	24	9.0	287	10.3	0.86	0.55, 1.33	0.88	0.56, 1.38
Daily		No nitrosatable drug exposure	85	71.4	1072	68.6	1.00	Referent	1.00	Referent
		Secondary amines	17	16.7	272	20.2	0.79	0.46, 1.35	0.85	0.49, 1.48
		Tertiary amines	14	14.1	246	18.7	0.72	0.40, 1.28	0.74	0.41, 1.35
		Amides	12	12.4	160	13.0	0.95	0.51, 1.77	1.03	0.54, 1.96
<Daily	P4P6	No nitrosatable drug exposure	85	74.6	697	76.8	1.00	Referent	1.00	Referent
		Secondary amines	17	16.7	107	13.3	1.30	0.74, 2.28	1.63	0.88, 3.01
		Tertiary amines	14	14.1	113	14.0	1.02	0.56, 1.85	1.29	0.67, 2.48
		Amides	8	8.6	65	8.5	1.01	0.47, 2.18	1.05	0.47, 2.36
Daily		No nitrosatable drug exposure	244	74.6	2870	73.0	1.00	Referent	1.00	Referent
		Secondary amines	42	14.7	642	18.3	0.77	0.55, 1.08	0.83	0.59, 1.17
		Tertiary amines	38	13.5	481	14.4	0.93	0.65, 1.33	0.95	0.66, 1.38
		Amides	30	11.0	323	10.1	1.09	0.74, 1.62	1.11	0.74, 1.66

Abbreviations: OR, odds ratio; CI, confidence interval; P1P3, first trimester; P4P6, second trimester

[a]Refers to timing of exposure for nitrosatable drugs and vitamin C supplement use.

[b]Percentages for no nitrosatable drug exposure are based on total participants with complete information, while percentages for secondary or tertiary amines and amides includes complete information for the given drug group and excludes other nitrosatable groups in the denominator.

[c]Crude and adjusted odds ratios include only cases and controls with complete information for drug exposures and covariates.

[d]Adjusted for maternal race/ethnicity, education, smoking, chronic hypertension, and study center.

(Table 4.3). Among women whose estimated daily intake of dietary vitamin C was 85 mg or more, a lower OR was observed for SGA in association with nitrosatable amide use during the second trimester of pregnancy (OR 0.84 [95% CI 0.50, 1.43]) than among women who took these drugs and lower estimated intake of dietary vitamin C (OR 1.39 [95% CI 0.84, 2.31]). On the other hand, higher ORs were noted for SGA in women who reported 85 mg or more of dietary vitamin C intake in conjunction with exposure to secondary amines during the first, second, and third trimester of pregnancy and nitrosatable amides during the first trimester of pregnancy. Restriction of analyses to full term case and control infants did not change the overall conclusion; however, nitrosatable amide use during the fifth month of pregnancy was associated with lower odds of SGA in women with 85 mg or more of daily dietary vitamin C intake (OR 1.09 [95% CI 0.56, 2.11]) compared with <85 mg of dietary vitamin C (OR 2.31 [95% CI 1.21, 4.42]) (data not shown). Exposure to amides during the sixth month of pregnancy was also associated with lower odds of SGA among women with higher intake of dietary vitamin C (OR 1.29 [95% CI 0.64, 2.58]) compared to those with less than 85mg of dietary vitamin C (OR 2.04 [95% CI 1.10, 3.81]) (data not shown).

With stratification by total vitamin C, a pattern of decreasing OR was observed for SGA among women with 85mg or more of dietary vitamin C and daily vitamin C supplementation in conjunction with exposures to secondary and tertiary amines during the second trimester of pregnancy (Table 4.4). The odds

Table 4.3 Effects of Maternal Nitrosatable Drug Exposures by Each Trimester of Pregnancy on Small-For-Gestational-Age Births Stratified by Dietary Vitamin C, National Birth Defects Prevention Study, 1997-2005

Dietary Vitamin C mg/day	Timing of drug exposure	Type of drug exposure	Cases		Controls		Unadjusted		Adjusted	
			No.	%[a]	No.	%[a]	OR[b]	95% CI	OR[b,c]	95% CI
<85	P1P3	No nitrosatable drug exposure	115	71.4	1329	67.3	1.00	Referent	1.00	Referent
		Secondary amines	21	15.4	345	20.6	0.70	0.44, 1.14	0.76	0.46, 1.24
		Tertiary amines	25	17.9	359	21.3	0.80	0.51, 1.26	0.87	0.54, 1.38
		Amides	15	11.5	211	13.7	0.82	0.47, 1.43	0.82	0.46, 1.44
≥85		No nitrosatable drug exposure	213	77.2	2238	75.6	1.00	Referent	1.00	Referent
		Secondary amines	33	13.4	383	14.6	0.91	0.62, 1.33	0.96	0.65, 1.44
		Tertiary amines	22	9.4	359	13.8	0.64	0.41, 1.01	0.70	0.44, 1.12
		Amides	21	9.0	232	9.4	0.95	0.60, 1.52	1.00	0.61, 1.62
<85	P4P6	No nitrosatable drug exposure	115	68.9	1329	69.4	1.00	Referent	1.00	Referent
		Secondary amines	24	17.3	343	20.5	0.81	0.51, 1.28	0.87	0.54, 1.39
		Tertiary amines	25	17.9	293	18.1	0.99	0.63, 1.55	1.00	0.63, 1.60
		Amides	21	15.4	170	11.3	1.43	0.87, 2.33	1.39	0.84, 2.31
≥85		No nitrosatable drug exposure	213	78.6	2238	76.6	1.00	Referent	1.00	Referent
		Secondary amines	34	13.8	404	15.3	0.88	0.61, 1.29	0.99	0.67, 1.47
		Tertiary amines	26	10.9	299	11.8	0.91	0.60, 1.40	0.96	0.62, 1.50
		Amides	17	7.4	219	8.9	0.82	0.49, 1.36	0.84	0.50, 1.43
<85	P7P9	No nitrosatable drug exposure	115	72.3	1329	71.4	1.00	Referent	1.00	Referent
		Secondary amines	19	14.2	314	19.1	0.70	0.42, 1.15	0.74	0.45, 1.24
		Tertiary amines	25	17.9	250	15.8	1.16	0.73, 1.82	1.18	0.74, 1.88
		Amides	19	14.2	158	10.6	1.39	0.83, 2.32	1.40	0.82, 2.37
≥85		No nitrosatable drug exposure	213	78.9	2238	78.8	1.00	Referent	1.00	Referent
		Secondary amines	31	12.7	361	13.9	0.90	0.61, 1.34	0.99	0.66, 1.49
		Tertiary amines	20	8.6	238	9.6	0.88	0.55, 1.42	0.91	0.56, 1.50
		Amides	21	9.0	169	7.0	1.31	0.81, 2.10	1.30	0.80, 2.13

Abbreviations: OR, odds ratio; CI, confidence interval; P1P3, first trimester; P4P6, second trimester; P7P9, third trimester

Table 4.3 Continued.

[a]Percentages for no nitrosatable drug exposure are based on total participants with complete information, while percentages for secondary or tertiary amines and amides includes complete information for the given drug group and excludes other nitrosatable groups in the denominator.

[b] Crude and adjusted odds ratios include only cases and controls with complete information for drug exposures and covariates and whose daily caloric intake was between 500 and 5000 kcal.

[c]Adjusted for maternal race/ethnicity, education, smoking, chronic hypertension, caloric intake, and study center.

Table 4.4 Effects of Maternal Nitrosatable Drug Exposures by Each Trimester of Pregnancy on Small-For-Gestational-Age Births Stratified by Total Vitamin C (Supplement and Diet), National Birth Defects Prevention Study, 1997-2005

Total Vitamin C[a]	Timing of exposure	Type of drug exposure	Cases		Controls		Unadjusted OR[c]	95% CI	Adjusted OR[c,d]	95% CI
			No.	%[b]	No.	%[b]				
Low/None	P1P3	No nitrosatable drug exposure	84	71.8	881	67.5	1.00	Referent	1.00	Referent
		Secondary amines	16	16.0	221	20.1	0.76	0.44, 1.32	0.80	0.45, 1.41
		Tertiary amines	17	16.8	245	21.8	0.73	0.42, 1.25	0.77	0.44, 1.35
		Amides	10	10.6	141	13.8	0.74	0.38, 1.47	0.73	0.37, 1.47
High/Daily		No nitrosatable drug exposure	55	71.4	632	70.0	1.00	Referent	1.00	Referent
		Secondary amines	13	19.1	150	19.2	1.00	0.53, 1.87	1.02	0.53, 1.97
		Tertiary amines	7	11.3	132	17.3	0.61	0.27, 1.37	0.61	0.26, 1.41
		Amides	7	11.3	90	12.5	0.89	0.39, 2.02	0.98	0.42, 2.28
Low/None	P4P6	No nitrosatable drug exposure	20	58.8	249	70.9	1.00	Referent	1.00	Referent
		Secondary amines	10	33.3	55	18.1	2.26	1.00, 5.11	**4.03[e]**	**1.49, 10.93**
		Tertiary amines	8	28.6	57	18.6	1.75	0.73, 4.17	2.68	0.95, 7.58
		Amides	1	4.8	26	9.5	0.48	0.06, 3.71	0.48	0.06, 4.04
		No nitrosatable drug exposure	146	77.3	1770	75.6	1.00	Referent	1.00	Referent
		Secondary amines	27	15.6	350	16.5	0.94	0.61, 1.43	1.01[e]	0.65, 1.57
		Tertiary amines	20	12.1	244	12.1	0.99	0.61, 1.62	1.04	0.63, 1.73
		Amides	10	6.4	180	9.2	0.67	0.35, 1.30	0.72	0.37, 1.41

Abbreviations: OR, odds ratio; CI, confidence interval; P1P3, first trimester; P4P6, second trimester; P7P9, third trimester

[a]Low/none refers to <85mg of dietary vitamin C intake and no vitamin C supplementation; high/daily refers to ≥85 mg of dietary vitamin C intake and daily vitamin C supplementation.

[b]Percentages for no nitrosatable drug exposure are based on total participants with complete information, while percentages for secondary or tertiary amines and amides includes complete information for the given drug group and excludes other nitrosatable groups in the denominator.

[c] Crude and adjusted odds ratios include only cases and controls with complete information for drug exposures and covariates and whose daily caloric intake was between 500 and 5000 kcal.

[d]Adjusted for maternal race/ethnicity, education, smoking, chronic hypertension, caloric intake, and study center.

[e]Significant additive interaction (95% confidence levels for RERI and/or AP exclude 0).

of SGA in association with secondary amine exposure during the second trimester of pregnancy were notably lower among women with 85 mg or more of dietary vitamin C intake and daily vitamin C supplementation (OR 1.01 [95% CI 0.65, 1.57]) compared to those with lower intake of dietary vitamin C and less than daily vitamin C supplementation (OR 4.03 [95% CI 1.49, 10.93]). Significant additive interaction (AP 0.57 [95% CI 0.22, 0.93]) was noted between total vitamin C and secondary amine exposure in relation to SGA.

Discussion

In this large population-based case-control study, the effects of vitamin C supplementation on associations between SGA and nitrosatable drugs appeared variable during the first and second trimester of pregnancy. Among women who took a daily supplement of vitamin C, lower ORs were observed for SGA with secondary and tertiary amines exposures during the second trimester of pregnancy compared to women with less than daily intake of vitamin C supplement. Conversely, higher ORs were noted for SGA in relation to nitrosatable amide use during the first trimester of pregnancy among women with daily vitamin C supplementation.

Although dietary vitamin C appeared to diminish the association between specific nitrosatable drugs and SGA, inconsistent findings were observed. Higher intake of dietary vitamin C slightly lowered the associations between tertiary amines during the third trimester of pregnancy, and amide drug exposure during the second and third trimester of pregnancy. The odds of SGA were

notably lower among mothers of full term case and control infants who reported 85 mg or more of dietary vitamin C intake in conjunction with nitrosatable amide exposure during the fifth and sixth month of pregnancy compared to those with lower intake of dietary vitamin C. In contrast, higher odds of SGA were observed with secondary amines exposure during the first, second, and third trimester of pregnancy and amide use during the first trimester in women with 85 mg or more of dietary vitamin C intake. Total vitamin C also modified the associations between nitrosatable drug and SGA, with lower ORs observed for SGA among women who reported higher intake of dietary vitamin C and daily vitamin C supplementation in conjunction with exposure to secondary and tertiary amines during the second trimester of pregnancy.

Limited epidemiologic studies have assessed the effect of vitamin supplementation on risk of SGA birth. In a prospective cohort study, Alwan et al. observed no significant association between daily vitamin supplementation during pregnancy and SGA. The ORs marginally decreased from 1.3 ([95% CI 0.8, 1.9]) to 0.9 ([95% CI 0.5, 1.7]) with vitamin supplement use from the first to third trimester of pregnancy.[176] These findings were consistent with a case control study conducted in Australia in which maternal use of vitamin supplement during the last month of pregnancy was associated with a slightly lower risk of SGA birth (OR 0.76 [95% CI 0.55, 1.05]).[18] Mathews et al. found total vitamin C (estimated from food and supplement) intake during early pregnancy to be positively associated with birthweight, with a mean difference of

100g between the lowest (< 55 mg/day) and highest thirds (≥ 98 mg/day) of intake.[15] Furthermore, Lee et al. noted that serum concentrations of maternal vitamin C measured during the second trimester of pregnancy were positively correlated with birth weight in full term births.[212] In the present study, we found that mothers who took less than daily supplement of vitamin C were more likely to have SGA births (OR 1.23 [95% CI 1.00, 1.50]) compared to those with daily vitamin C supplementation during the first trimester of pregnancy. Less than daily use of vitamin C supplement during the second trimester of pregnancy was significantly associated with SGA (OR 1.44 [95% CI 1.17, 1.78]).

Vitamin C is known to inhibit N-nitroso compound formation when administered concurrently with a nitrosatable precursor.[172] However, in the NBDPS, participants were not questioned about the specific timing of dietary or supplemental vitamin C in relation to nitrosatable drug use. Hence, any effect modification observed may not be due to the effect of vitamin C itself but possibly due to other nutrients such as vitamin E commonly found in prenatal or multivitamins or healthy behaviors correlated with vitamin C supplementation or higher intake of dietary vitamin C.

The study had several other limitations. Misclassification of foods consumed during pregnancy might have occurred. In the NBDPS, women were interviewed about the frequency of foods consumed a year prior to conception. Because the same period of dietary assessment was used for all NBDPS participants, the misclassification would most likely be non-differential with

respect to the outcome (SGA), and it would have minimal effect on any association observed with nitrosatable drugs by dietary vitamin C. Furthermore, Cuco et al. found no significant difference in average consumption of vegetables as measured before pregnancy and in weeks 6, 10, 26, and 38 of pregnancy.[195] Findings from another study indicated no change in mean intake of vegetables but a difference in fruit intake was noted between the first and third trimester of pregnancy. Strong correlations were also observed between the first and third trimester consumption of vegetables and fruits. [13] Vegetables and fruits are common sources of vitamin C.

Another limitation was the potential maternal recall bias of drug exposures during pregnancy. Studies have found little evidence for differential recall of several drugs that have nitrosatable drug components including analgesics, antibiotics, and antinauseants between women with normal or adverse pregnancy outcomes.[196, 197] In the NBDPS, participants were questioned about medications by indication of use and drug names. This two-level approach has shown to be more accurate than an open ended questionnaire.[198, 199] Also, women were not aware of the nitrosatable drug components in the drugs. The reported drugs were later classified into secondary amines, tertiary amines, and amides depending on their nitrosatability. Further, analyses of the study included exposure data for all NBDPS controls who had births without congenital malformations; hence it is

less likely that recall bias may have occurred than what might be expected among mothers of babies without major birth defects.

Moreover, smaller sample sizes were available for some of the models examining the association between nitrosatable drugs and SGA by supplemental or dietary vitamin C. Among women who reported less than daily use of vitamin C supplement, the minimum detectable ORs were 1.5 and 1.7, respectively, with tertiary amine and amide exposures during the first trimester of pregnancy. But in women with secondary amines exposure during the second trimester, the smallest detectable OR was 2.0 at the same power level. Additionally, in women with lower intake of dietary vitamin C (<85 mg/day), we could detect ORs between 1.8 and 2.0 with 80% power in relation to tertiary amine and amide drug exposure. Findings were not presented for the association between SGA and nitrosatable drugs stratified by vitamin C supplementation during the third trimester of pregnancy due to incomplete data available on vitamin C supplement use during this period.

In conclusion, findings of this study suggest that supplemental and dietary vitamin C intake during pregnancy presented variable effects on the associations between nitrosatable drugs and SGA depending on trimester of use and type of nitrosatable drug. Daily vitamin C supplementation in combination with higher dietary vitamin C intake (≥85 mg/day) also modified the association between nitrosatable drugs and SGA. Based on what is known regarding the role of vitamin C in inhibiting N-nitroso compound formation when given together with a

nitrosatable compound, more research is needed to examine the relation between SGA and nitrosatable drugs with respect to timing of vitamin C supplement use and nitrosatable drugs exposure.

5. SUMMARY AND CONCLUSIONS

Summary

The causes and mechanism of SGA are not well understood. Numerous environmental contaminants including maternal exposure to air pollutants and tobacco smoke have been associated with SGA but evidence is limited on the association between nitrate and nitrite exposure from diet, nitrosatable drugs and SGA. Nitrate is a ubiquitous contaminant in food and water. Certain medications, which contain nitrosatable amines (secondary or tertiary amines) or amides can react with nitrosating agents such as nitrite in the acidic environment of the stomach to form N-nitroso compounds.[24] Evidence from animal models suggested that combined exposure to nitrite and a nitrosatable compound were associated with reduced birthweight in offspring.[92] No published study has examined the association between maternal exposure to nitrosatable drugs and dietary nitrate/nitrite intake and SGA.

In this population-based case-control study, we found that maternal exposure to nitrosatable drugs and their specific functional groups (secondary amines, tertiary amines, or amides) any time during pregnancy was not generally associated with SGA. However, maternal use of drugs classified as nitrosatable amides during the third trimester of pregnancy was significantly associated with SGA. Higher ORs were noted for SGA in relation to amide drug use during the eighth and ninth month of pregnancy. Furthermore, these associations were stronger when analyses were restricted to full term case- and

control-infants, and exposure to amides during the sixth month of pregnancy was also associated with SGA in full term births.

Dietary consumption accounts for a significant portion of daily nitrite exposure. Ingested nitrates are converted to nitrites in the saliva (approximately 5%) and a portion of the nitrites is converted to nitric oxide in the acidic environment of the stomach.[20, 21] Studies have detected elevated nitric oxide levels in cord blood and placental tissue of pregnancies with intrauterine growth retardation.[83, 84] In the present study, higher consumption of dietary nitrates, nitrites, and total nitrites were not significantly associated with SGA. Moreover, we examined if higher intake of dietary nitrites and total nitrites strengthened the associations between nitrosatable drugs and SGA; but a reverse trend was noted with modest associations observed for the highest tertile of dietary nitrite intake. Women with the lowest estimated intake of nitrite and total nitrites were more likely to have a SGA offspring if they reported exposure to drugs classified as nitrosatable amides during the third trimester of pregnancy. A lower intake of dietary nitrites from animal sources in conjunction with exposure to amide drugs was significantly associated with SGA, but consumption of plant source of nitrites had no effect on risk of SGA births.

Vitamin C is known to inhibit N-nitroso compound formation when administered concurrently with a nitrosatable precursor. We examined whether supplemental and dietary vitamin C diminished the association between nitrosatable drugs and SGA. The effects of vitamin C supplementation on the

associations between SGA and nitrosatable drugs appeared variable during the first and second trimester of pregnancy. Among women who took a daily supplement of vitamin C, higher odds of SGA were observed in conjunction with exposure to nitrosatable amides during the first trimester of pregnancy compared to women exposed to these drugs and less than daily intake of vitamin C supplement. Conversely, lower ORs were noted for SGA in women with daily vitamin C supplementation in conjunction with exposure to secondary amines during the second trimester of pregnancy than ORs for women who took these drugs and less than daily intake of a vitamin C supplement.

Dietary vitamin C presented inconsistent findings on the association between SGA and nitrosatable drug use during pregnancy. Higher intake of dietary vitamin C (≥85 mg/day) slightly lowered the associations between SGA and amide drug use during the second trimester of pregnancy. On the other hand, higher odds of SGA were observed with amide use during the first trimester in women with 85 mg or more of dietary vitamin C intake. Total vitamin C also modified the associations between nitrosatable drug and SGA. The odds of SGA in association with secondary and tertiary amines during the second trimester of pregnancy were lower among women who reported higher intake of dietary vitamin C (≥85 mg/day) and daily vitamin C supplementation compared to women with <85 mg of dietary vitamin C and less than daily use of vitamin C supplement.

Conclusions

Prenatal use of drugs that have nitrosatable components did not appear to increase the odds of delivering a SGA infant except for a few notable exceptions. Higher consumption of dietary sources of nitrates and nitrites was not found to be associated with SGA, nor did higher nitrite intake strengthen the association between nitrosatable drugs and SGA. Exposure to other environmental toxicants and other sources of nitrates such as drinking water might contribute to fetal growth restriction. Drinking water nitrates below the maximum contaminant level of 10 mg/L have been associated with intrauterine growth retardation.[213] However, the combined effect of nitrate exposure from diet and drinking water on the relation between nitrosatable drugs and SGA needs to be further examined.

Daily use of supplements containing vitamin C in conjunction with higher intake of dietary vitamin C slightly lowered the odds of SGA in relation to nitrosatable drug use during pregnancy. Women should be recommended to take a daily supplement containing vitamin C and to increase the intake of vegetables and fruits; these health habits may reduce the risk of SGA associated with nitrosatable drug use during pregnancy; many of such drugs being over the counter. Although adverse health outcomes associated with being born SGA are well known, limited research has focused on the determinants of SGA, some of which may be modifiable.

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